

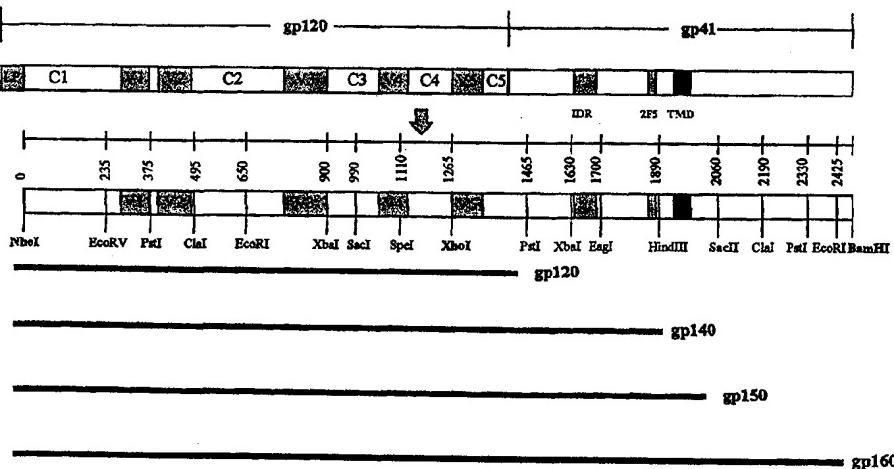
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(54) Title: METHOD FOR PRODUCING A NUCLEOTIDE CONSTRUCT WITH OPTIMISED CODONS FOR AN HIV GENETIC VACCINE BASED ON A PRIMARY, EARLY HIV ISOLATE AND SYNTHETIC ENVELOPE BX08 CONSTRUCTS

Synthetic BX08 Env
Strategy for building the full-length gp160 and derived truncated forms

**(57) Abstract**

The present invention relates to a method for producing a nucleotide sequence construct with optimized codons for an HIV genetic vaccine based on a primary, early HIV isolate. Specific such nucleotide sequence construct are the synthetic envelope BX08 constructs. The invention further relates to the medical use of such constructs for the treatment and prophylaxis of HIV through DNA vaccine and for diagnostics.

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Method for producing a nucleotide sequence construct with optimised codons for an HIV genetic vaccine based on a primary, early HIV isolate and synthetic envelope BX08 constructs.

5 Field of the invention

The invention relates to a DNA vaccine against HIV, which is designed from a clinical primary isolate. One aspect of the invention relates to a method of producing a nucleotide sequence construct, in a preferred aspect based on a cassette system, the nucleotide sequence construct being used as a DNA vaccine. The method can, for example, lead to the disclosed synthetic BX08 HIV-1 envelope vaccine nucleotide sequence construct, designed to generate suitable DNA vaccines against HIV, specifically HIV-1. Furthermore, the invention can be used for the production of recombinant protein antigens.

Background of the invention

15 There is an urgent need for new vaccine strategies against HIV. One such new promising strategy is called genetic immunisation or DNA vaccine (Webster et al 1997). Some of the advantages of a DNA vaccine against HIV is the induction of Th cell activation, induction of antibodies also against conformational dependent epitopes, and the induction of cellular immunity. So far, most DNA vaccine envelope genes tried, have been from tissue culture
20 adapted virus strains (Boyer et al 1997) that often differs in several aspects from primary clinical isolates (such as early isolates) e.g. in co-receptor usage (Choe et al 1996, Dragic et al 1997).

One disadvantage in HIV envelope based DNA vaccines may be the intrinsic relatively low
25 expression which is regulated by the Rev expression. This may prevent an optimal investigation of the vaccines in small animal models like mice where Rev is functioning suboptimally. Recently it has been shown using the tissue culture adapted HIV-1 MN strain, that an exchange of the HIV codon usage to that of highly expressed mammalian genes greatly improves the expression in mammalian cell lines and renders the HIV expression Rev
30 independent (Haas et al 1996). Additionally, it is known that rare codons cause pausing of the ribosome, which leads to a failure in completing the nascent polypeptide chain and a uncoupling of transcription and translation. Pausing of the ribosome is thought to lead to exposure of the 3' end of the mRNA to cellular ribonucleases.

The world-wide spread of HIV-1 has presently resulted in 8,500 new infections daily and AIDS is now the number 1 cause of death among US males (and number 3 among US females) aged 25-40 years. The epidemic hot-spots now include Eastern Europe, India and

5 South East Asia and southern Africa. The attempts to solve this world-wide problem involve education, prevention, treatment and vaccine development. Affordable protective vaccines represent the best solution to the world-wide problem of infection with HIV-1. Induction of virus neutralising antibodies is one of the key components in vaccine development. Several recombinant envelope vaccines have been tested in humans and animals, however, they

10 seem unable to induce sufficient protection. In this respect DNA vaccination may provide a different and more natural mode of antigen presentation. It is hoped that the immune responses induced by such DNA vaccines could aid in limiting virus replication, slowing disease progression or preventing occurrence of disease. Unfortunately many HIV envelope vaccines induce only moderate levels of antibodies. This could in part be due to limitations in

15 expression, influenced by regulation by the Rev protein and by a species-specific and biased HIV codon usage. Also the virus variability is considered a barrier for development of antibody based vaccines and thus a tool for more easy producing of closely related vaccine variants is needed.

20 It has been suggested to improve the immunogenicity and antigenicity of epitopes by certain mutations in the envelope gene. An elimination of certain immune dominant epitopes (like V3) could render less immune dominant but more relevant, conserved, or hidden epitopes more immunogenic (Bryder et al 1999). Also elimination of certain N-linked glycosylation sites could improve the exposure of relevant epitopes and increase the immunogenicity of

25 those epitopes. Thus, it is possible that elimination of the glycosylation sites in V1 and V2 may in a more favourable way expose neutralising epitopes (Kwong et al 1998, Wyatt et al 1998). The HIV envelope contains putative internalisation sequences in the intracellular part of gp41 (Sauter et al 1996). Thus it would be relevant to eliminate and/or mutate the internalisation signals in a membrane bound HIV envelope vaccine gene to increase the

30 amount of surface exposed vaccine derived HIV glycoproteins as gp150. Since the antibody response, that is measured and calculated in titers, is improved by adding the secreted gp120 as opposed to adding the membrane bound form (Vinner et al 1999), it could be advantageous to express the vaccine as a secreted gp120 or a secreted gp140. This would include important parts of gp41, such as the 2F5 neutralising linear epitope (Mascola et al

35 1997).

Summary of the invention

Our suggested solution to the problems described above is to design DNA envelope vaccines from a clinical primary isolate with Rev-independent high expression in mammals, that is built as a cassette for easy variant vaccine production.

5

A method of producing a nucleotide sequence construct with codons from highly expressed mammalian proteins based on a cassette system coding for an early, primary HIV envelope is described. The method comprises the steps of direct cloning of an HIV gene, derived from a patient within the first 12 months of infection, thereby obtaining a first nucleotide sequence;

- 10 designing a second nucleotide sequence utilising the most frequent codons from mammalian highly expressed proteins to encode the same amino acid sequence as the first nucleotide sequence; redesigning the second nucleotide sequence so that restriction enzyme sites surround the regions of the nucleotide sequence encoding functional regions of the amino acid sequence and so that selected restriction enzyme sites are removed, thereby obtaining
- 15 a third nucleotide sequence encoding the same amino acid sequence as the first and the second nucleotide sequence; redesigning the third nucleotide sequence so that the terminals contain convenient restriction enzyme sites for cloning into an expression vehicle; producing snuts between restriction enzyme sites as well as terminal snuts and introducing snuts into an expression vehicle by ligation. The nucleotide sequence construct obtained by this
- 20 method uses the mammalian highly expressed codons (figure 1) and renders the envelope gene expression Rev independent and allows easy cassette exchange of regions surrounded by restriction enzyme sites that are important for immunogenicity, function, and expression.

- 25 The method can, for example, lead to the disclosed synthetic, Rev-independent, clinical (such as early), primary HIV-1 envelope vaccine gene, built as a multi cassette. From the sequence of the envelope of the HIV-1 BX08 isolate (personal communication from Marc Girard, Institute Pasteur, Paris), the present inventors have designed a synthetic BX08 HIV-1 envelope vaccine nucleotide sequence construct.

30

With the great diversity of envelopes in HIV among different patients and within one patient, it would be of advantage to vaccinate with several envelope variants, all being highly expressed. To avoid synthesising several full length envelopes, it is much easier to exchange relevant parts of an envelope cassette to various strains in a multivalent vaccine.

35

- Whether it is the disclosed synthetic BX08 nucleotide sequence construct, or any of the nucleotide sequence constructs obtained by the method, they are designed to generate suitable DNA vaccines against HIV, specifically HIV-1. In this case the mammal, preferably a human being, is inoculated with the nucleotide sequence construct in an expression vehicle
- 5 and constitutes a host for the transcription and translation of the nucleotide sequence construct. The nucleotide sequence constructs of the present invention can furthermore be used for the production of recombinant protein antigens. In this case the nucleotide sequence construct is placed in an expression vehicle and introduced into a system (e.g. a cell-line), allowing production of a recombinant protein with the same amino acid sequence.
- 10 The recombinant protein is then isolated and administered to the mammal, preferably a human being. The immune system of the mammal will then direct antibodies against epitopes on the recombinant protein. The mammal, preferably a human being, can thus be primed or boosted with DNA and/or recombinant protein obtained by the method of the invention.
- 15 A relevant HIV DNA vaccine can potentially be used not only as a prophylactic vaccine, but also as a therapeutic vaccine in HIV infected patients, e.g. during antiviral therapy. An HIV specific DNA vaccine will have the possibility to induce or re-induce the wanted specific immunity and help the antiviral therapy in limiting or even eliminating the HIV infection. The
- 20 immunogenicity and antigenicity of epitopes in the envelope can be improved by certain mutations in the envelope gene. The cassette system allows for easy access to the relevant parts of the envelope gene, and thereby eased efforts in the process of genetic manipulation. Some suggested mutations are: an elimination of certain immune dominant epitopes (like V3); elimination of certain N-linked glycosylation sites (like glycosylation sites around V2);
- 25 elimination and/or mutation of the nucleotide sequence encoding the internalisation signals in the cytoplasmic part of a membrane bound HIV envelope to increase the amount of surface exposed vaccine derived HIV glycoproteins; elimination or mutation of the cleavage site between gp120 and gp41; with introduced mutations in gp41 for preserving conformational epitopes.
- 30 Table 1 below, lists the nucleotide sequence constructs of the invention by the names used herein, as well as by reference to relevant SEQ ID NOs of DNA sequences, and the amino acid sequence encoded by the DNA sequence in the preferred reading frame. It should be noted, that the snut name consist of the number of the approximate position for the end of
- 35 the snut and the restriction enzyme used to cleave and/or ligate that end of the snut.

Table 1 List of names of nucleotide sequence constructs (Snuts (S) and Pieces (P)) with reference to SEQ ID NO for the nucleotide sequence and protein sequence.

Name	Nucleotide SEQ ID NO:	Protein SEQ ID NO:
S _{O-N-Lang}	1	2
S _{235EcoRV}	3	4
S _{375PstI}	5	6
S _{495Clal}	7	8
S _{650-720EcoRI}	9	10
S _{900XbaI}	11	12
S _{990SacI}	13	14
S _{1110SpeI}	15	16
S _{1265Xhol}	17	18
S _{1265gp120}	19	20
S _{1265gp160}	21	22
S _{1465PstI}	23	24
S _{1465PstI cys}	25	26
S _{1630XbaI}	27	28
S _{1700EagI}	29	30
S _{1890HindIII}	31	32
S _{2060SacII}	33	34
S _{2190Clal}	35	36
S _{2330PstI}	37	38
S _{2425ES}	39	40
P ₁	41	42
P ₂	43	44
P ₃	45	46
P _{3GV1}	47	48
P _{3 GV1V2}	49	50
P _{3GV2}	51	52
P _{4gp160}	53	54
P _{4gp150}	55	56
P _{4gp140}	57	58
P ₅	59	60
P _{8gp160}	61	62
P _{8gp150}	63	64
P _{8gp140}	65	66
synBX08-140	67	68
synBX08-150	69	70
synBX08-160	71	72
synBX08-120	73	74
synBX08-41	75	76

Detailed disclosure of the invention

One aspect of the present invention relates to a method for producing a nucleotide sequence construct coding for an HIV gene. The nucleotide sequence construct is produced as a cassette system consisting of snuts. A snut (S) is a nucleotide sequences construct between

- 5 restriction enzyme cleavage sites comprising the minimal entity of the cassette system.

First an HIV gene is obtained from a patient within the first 12 months of infection. The term

HIV should be understood in the broadest sense and include HIV 1 and HIV 2. It is possible to determine the period in which the infection has taken place with an accuracy depending

- 10 on the frequency of the blood tests taken from the patient. For example, patients suffering from various diseases such as lack of certain factors in their blood or hepatitis have their blood tested on a regular basis making it possible to determine the period in which the infection has taken place. Apart from patients with diseases wherein blood tests are used to monitor the course of the disease, other groups of patients have blood tests taken, e.g. blood

- 15 donors. Unfortunately, humans are still infected due to transfer of virus in blood samples, medical equipment, etc., making it possible to determine the date where the infection has taken place within the time frame of a few days. The importance of obtaining the virus early in the course of the infection is due to the known fact that many early isolates share the common feature of staying relatively constant in their envelope sequences (Karlsson et al.,

- 20 1998). As these early isolates may share cross-reactive antibody- and/or T-cell epitopes a vaccine based on such early isolates would have a better chance of inducing immune response to shared epitopes of the virus. It is believed that an early, directly cloned virus isolate will share immunogenic sites with other early virus isolates seen during an HIV infection, so that if a mammal generates antibodies and/or T-cells directed against these

- 25 epitopes, the transferred virus will be eliminated prior to the extensive mutations that may occur after approximately 12 months of infection. Thus, the virus should be isolated as early as possible, that is within the first 12 months of infection, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0.5 month after infection.

- 30 The HIV gene for genetic vaccine is preferably cloned directly from viral RNA or from proviral DNA. Direct cloning in this application stands for the virus not being multiplied in stable cell lines *in vitro*. It is presently expected that passing the virus through a stable cell line will promote mutation in the virus gene. It is particularly preferred not to pass the virus through cells lines selecting for viruses with CXCR4 receptor usage. Direct cloning also includes
- 35 multiplication of virus in e.g. PBMC (peripheral blood mononuclear cells) since all virus can

multiply in PBMC, and this type of multiplication generally does not select for CXCR4 receptor usage. Multiplication of virus is often necessary prior to cloning. Preferably cloning is performed directly on samples from the patient. In one embodiment of the invention, cloning is performed from patient serum. The cloning is then performed directly on the HIV 5 virus, that is on RNA. In another embodiment of the invention cloning, is performed from infected cells. The cloning is then performed on HIV virus incorporated in the genes in an infected cell (e.g. a lymphocyte), that is on DNA. In the latter case the virus might be a silent virus, that is a non-replicating virus. To evaluate if the virus is silent, capability of multiplication in e.g. PBMC is tested.

10

Cloning is a technique well known to a person skilled in the art. A first nucleotide sequence is hereby obtained. In another aspect of the invention, the first nucleotide sequence, sharing the properties mentioned with direct cloning, is obtained by other means. This could be from a database of primary isolates or the like.

15

Based on the first nucleotide sequence, the amino acid sequence encoded by said nucleotide sequence is determined. A second nucleotide sequence encoding the same amino acid sequence is then designed utilising the most frequent codons from highly expressed proteins in mammals (e.g. figure 1 presenting the most frequent codons from 20 highly expressed proteins in humans).

25

Presently, it appears that the usage of the most frequent codons from mammalian highly expressed proteins has two advantages: 1) the expression is Rev independent; 2) the level of expression is high. The Rev independence is especially advantageous when performing experiments in mice where the Rev system is functioning sub-optimally. For the use in human vaccine, Rev independence and high expression are important to increase the amount of antigen produced. The determination of the codons for high expression is in this context based on the statistics from human highly expressed proteins (Haas, Park and Seed, 1996 hereby incorporated by reference). It is contemplated that the expression of a protein 30 can be even higher, when current research in binding between codon (on the mRNA) and anticodon (on the tRNA) reveals codons with optimal binding capabilities, and when interactions in-between codons and/or in-between anticodons are known.

35

The second nucleotide sequence designed utilising optimised codons is then redesigned to obtain a third nucleotide sequence. The purpose of the redesigning is to create unique restriction enzyme sites around the nucleotide sequence encoding functional regions of the

- amino acid sequence. By having unique restriction enzyme sites around the nucleotide sequence encoding functional regions of the amino acid sequence, the nucleotide sequence encoding functional regions of the amino acid sequence can easily be isolated, changed, and re-inserted. Examples of functional regions of the amino acid sequence are transmembrane spanning regions, immunodominant regions, regions with antibody cross reacting domains, fusion domains and other regions important for immunogenicity and expression such as variable region 1 (V1), variable region 2 (V2), variable region 3 (V3), variable region 4 (V4) and variable region 5 (V5).
- 10 It is important to select the restriction enzymes sites with care. By changing the second nucleotide sequence to insert restriction enzyme sites around the nucleotide sequence encoding functional regions of the amino acid sequence, the third nucleotide sequence must still code for the same amino acid sequence as the second and first nucleotide sequence do. Thus, if necessary, the second nucleotide sequence is redesigned by changing from
- 15 optimised codons to less optimal codons. It is understood, that the restriction enzyme sites around the nucleotide sequence encoding functional regions of the amino acid sequence should preferably be placed in the terminal region of the nucleotide sequence encoding functional regions of the amino acid sequence. That is preferably outside the nucleotide sequence encoding functional regions of the amino acid sequence, such as 90 nucleotides
- 20 away, e.g. 81, 72, 63, 54, 45, 36, 27, 21, 18, 15, 12, 9, 6, 3 nucleotides away, but could also be inside the nucleotide sequence encoding functional regions of the amino acid sequence, such as 54, 45, 36, 27, 21, 18, 15, 12, 9, 6, 3 nucleotides inside the nucleotide sequence encoding the functional region of the amino acid sequence.
- 25 The type of restriction enzyme sites allowed is determined by the choice of expression vector. In certain cases, the number of restriction enzyme sites is limited and it is hard, if not impossible, to place unique restriction enzyme sites around all the nucleotide sequences coding for functional regions of the amino acid sequence. This problem can be solved by dividing the entire nucleotide sequence into pieces, so that each piece comprises only
- 30 unique restriction enzyme sites. Modifications to each of the piece is performed separately prior to assembly of the pieces. It is preferred that the nucleotide sequence is divided into 9 pieces. In another aspect, the nucleotide sequence is divided into 8 pieces, or 7, or 6, or 5, or 4, or 3, or 2 pieces. It is especially preferred that the nucleotide sequence is divided into 3 pieces.

Thus, the redesign of the second nucleotide sequence is an interaction between the choice of cloning vector, expression vector, selection of restriction enzyme sites, dividing into pieces, and exchange of codons to insert restriction enzyme sites. In a preferred embodiment of the present invention the cloning vector is Bluescript allowing the restriction enzyme sites chosen from the group consisting of: *EagI*, *MluI*, *EcoRV*, *PstI*, *ClaI*, *EcoRI*, *XbaI*, *SacI*, *SpeI*, *XhoI*, *HindIII*, *SacII*, *NotI*, *BamHI*, *SmaI*, *SalI*, *DraI*, *KpnI*. If other cloning vectors are chosen, other restriction enzyme sites will be available as known by the person skilled in the art.

- 10 As a part of the redesigning of the second nucleotide sequence, selected restriction enzyme sites may be removed. The selected restriction enzyme sites to be removed are those sites that are sites of the same type as the ones already chosen above and that are placed within the same piece. The removal of these restriction enzyme sites is performed by changing from optimised codons to less optimal codons, maintaining codons for the same amino acid
- 15 sequence.

- The third nucleotide sequence is redesigned so that the terminal snuts contain convenient restriction enzyme sites for cloning into an expression vehicle. The expression "vehicle" means any nucleotide molecule e.g. a DNA molecule, derived e.g. from a plasmid,
- 20 bacteriophage, or mammalian or insect virus, into which fragments of nucleic acid may be inserted or cloned. An expression vehicle will contain one or more unique restriction enzyme sites and may be capable of autonomous replication in a defined host or vehicle organism such that the cloned sequence is produced. The expression vehicle is an autonomous element capable of directing the synthesis of a protein. Examples of expression vehicles are
 - 25 mammalian plasmids and viruses, tag containing vectors and viral vectors such as adenovirus, vaccinia ankara, adenoassociated virus, canarypox virus, simliki forest virus (sfv), Modified Vaccinia Virus Ankara (MVA), and simbis virus. In one embodiment of the invention, the expression vector contains tag sequences. In another embodiment of the invention a bacteria is transformed with an expression plasmid vector and the bacteria is
 - 30 then delivered to the patient. Preferred expression vehicles are simliki forest virus (sfv), adenovirus and Modified Vaccinia Virus Ankara (MVA).

- The snuts are produced by techniques well known by the person skilled in the art. The preferred method for synthesising snuts, is herein referred to as "the minigene approach" 35 wherein complementary nucleotide strands are synthesised with specific overhanging sequences for annealing and subsequent ligation into a vector. This can be performed with

two sets of complementary nucleotide strands, or with three sets of complementary nucleotide strands. The minigene approach minimises the known PCR errors of mismatches and/or deletions, which may occur due to hairpins in a GC rich gene with mammalian highly expressed codons. In figures 10-21, the production of a representative selection of snuts is 5 illustrated.

For the production of long snuts, that is snuts of more than about 240 nucleotides, the technique of overlapping PCR is preferred as illustrated in figure 8. Herein two nucleotide strands about 130 nucleotides long with an overlap are filled to obtain a double strand, which 10 is subsequently amplified by PCR.

For the production of multiple snuts with a length of less than about 210 nucleotides, one preferred technique is normal PCR. In a preferred production technique the snuts are synthesised with the same 5' flanking sequences and with the same 3' flanking sequences, 15 as illustrated in figure 9. The advantages of this approach is, that the same PCR primer set can be used for amplification of several different snuts.

As known by the person skilled in the art, special conditions have to be used for each individual PCR reaction and it should be optimised to avoid inherent problems like deletions 20 mismatches when amplifying GC rich genes from synthetic ssDNA material. Whichever of the above mentioned techniques are used, it is well known by the person skilled in the art, that it will be necessary to correct unavoidable mismatches produced either due to the nucleotide strand synthesis material and/or the PCR reaction. This can be performed by site directed mutagenesis techniques.

25

After the various snuts have been produced, they are assembled into pieces and subsequently into the complete gene. Methods for assembly (such as ligation) are well known by the person skilled in the art.

30

In a preferred embodiment of the present invention the HIV gene encodes the entire HIV envelope. It is understood that the HIV envelope can be the full length envelope gp160 as well as shorter versions such as gp150, gp140, and gp120 with or without parts of gp41.

35 As will be known by the person skilled in the art, the HIV is divided into several groups. These groups presently include group M, group O, and group N. Further, the HIV is divided

into subtypes A, B, C, D, E, F, G, H, I, and J. In the present invention subtype B is preferred due to the high prevalence of this subtype in the Western countries.

The determination of groups and subtypes is based on the degree of nucleotide sequence identity in the envelope gene is presently defined as follows: If the sequence identity is more than 90% the viruses belong to the same subtype; If the sequence identity is between 80% and 90% the viruses belong to the same group. If the sequence identity is less than 80% the viruses are considered as belonging to different groups.

- 10 One aspect of the invention relates to a nucleotide sequence construct in isolated form which has a nucleotide sequence with the general formula (I), (II), (III), or (IV)

(I) $P_1-S_{495Clal}-S_{650-720EcoRI}-P_2-S_{1265gp120}$

(II) $P_1-S_{495Clal}-S_{650-720EcoRI}-P_2-S_{1265Xhol}-S_{1465PstI}-P_{4gp140}$

(III) $P_1-S_{495Clal}-S_{650-720EcoRI}-P_2-S_{1265Xhol}-S_{1465PstI}-P_{4gp150}$

- 15 (IV) $P_1-S_{495Clal}-S_{650-720EcoRI}-P_2-S_{1265Xhol}-S_{1465PstI}-P_{4gp160}-S_{2060SacII}-P_5$

wherein P_1 designates the nucleotide sequence SEQ ID NO:41, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 90% thereto;

wherein $S_{495Clal}$ designates the nucleotide sequence SEQ ID NO: 7, a nucleotide sequence

- 20 complementary thereto, or a nucleotide sequence with a sequence identity of at least 95% thereto;

wherein $S_{650-720EcoRI}$ designates the nucleotide sequence SEQ ID NO: 9, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 95% thereto;

- 25 wherein P_2 designates the nucleotide sequence SEQ ID NO: 43, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;

wherein $S_{1265gp120}$ designates the nucleotide sequence SEQ ID NO: 19, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at

- 30 least 70% thereto;

wherein $S_{1265Xhol}$ designates the nucleotide sequence SEQ ID NO: 17, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 80% thereto;

wherein $S_{1465PstI}$ designates the nucleotide sequence SEQ ID NO: 23, a nucleotide sequence

- 35 complementary thereto, or a nucleotide sequence with a sequence identity of at least 90% thereto;

- wherein P_{4gp140} designates the nucleotide sequence SEQ ID NO: 57, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;
- wherein P_{4gp150} designates the nucleotide sequence SEQ ID NO: 55, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;
- wherein P_{4gp160} designates the nucleotide sequence SEQ ID NO: 53, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;
- 10 wherein $S_{2060SacII}$ designates the nucleotide sequence SEQ ID NO: 33, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 98% thereto; and
- wherein P_5 designates the nucleotide sequence SEQ ID NO: 59, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85%
- 15 thereto.

The design of the parent synthetic BX08 gp160 envelope cassette gene with its variant length genes gp150, gp140, gp120 is outlined in figure 2.

- 20 The nucleotide sequence construct with the formula (I)
- (I) $P_1-S_{495ClaI}-S_{650-720EcoRI}-P_2-S_{1265gp120}$
(visualised in figure 3) (SEQ ID NO: 73) codes for the amino acid sequence of gp120 (SEQ ID NO: 74). This amino acid sequence is the part of the HIV envelope that is secreted. Thus, it contains the immunogenic epitopes without being bound to the cell membrane. This is of particular advantage if the nucleotide sequence construct is used for production of recombinant antigens or for a DNA vaccine as the antibody immune response may be higher to secreted versus membrane bound HIV antigens.

The nucleotide sequence construct with the formula (II)

- 30 (II) $P_1-S_{495ClaI}-S_{650-720EcoRI}-P_2-S_{1265Xhol}-S_{1465PstI}-P_{4gp140}$
(visualised in figure 4) (SEQ ID NO: 67) codes for the amino acid sequence of gp140 (SEQ ID NO: 68). This amino acid sequence encodes the gp120 and the extracellular part of the gp41 protein. The amino acid sequence is secreted due to the lack of the transmembrane spanning region. This is of particular advantage if the nucleotide sequence construct is used for production of recombinant antigens as the immunogenic and/or antigenic epitopes in the extracellular part of gp41 are included and is of particular advantage for a DNA vaccine as

the antibody immune response may be higher to secreted gp120 versus membrane bound HIV antigens.

The nucleotide sequence construct with the formula (III)

- 5 (III) $P_1-S_{495}ClaI-S_{650-720}EcoRI-P_2-S_{1265}XhoI-S_{1465}PstI-P_{4gp150}$
(visualised in figure 5) (SEQ ID NO: 69) codes for the amino acid sequence of gp150 (SEQ ID NO: 70). This amino acid sequence contains all of the envelope protein gp160 except the c-terminal tyrosin containing internalisation signals in the intracellular part of gp41. The membrane bound surface expression of the amino acid sequence is thereby maintained and
10 enhanced. Mimicking the organisation of the native epitope conformation may by expected, making this nucleotide sequence construct of particular advantage if the nucleotide sequence construct is used as a vaccine.

The nucleotide sequence construct with the formula (IV)

- 15 (IV) $P_1-S_{495}ClaI-S_{650-720}EcoRI-P_2-S_{1265}XhoI-S_{1465}PstI-P_{4gp160}-S_{2060}SacII-P_5$
(visualised in figure 6) (SEQ ID NO: 71) codes for the amino acid sequence of gp160 (SEQ ID NO: 72) i.e. the entire envelope.

The nucleotide sequence construct designated P_1 comprises the nucleotide sequence
20 encoding the amino acid sequence in the first variable region (V1) and the amino acid sequence in the second variable region (V2). In one embodiment of the invention the first variable region is surrounded by EcoRV and PstI restriction enzyme sites, and the second variable region is surrounded by PstI and Clal restriction enzyme sites but as stated above, the choice of restriction enzyme sites can alter.

25

The nucleotide sequence construct designated $S_{650-720}EcoRI$ comprises the nucleotide sequence encoding the amino acid sequence in the third variable region (V3). In one embodiment of the present invention $S_{650-720}EcoRI$ is characterised by the restriction enzyme sites EcoRI and XbaI in the terminals.

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The nucleotide sequence construct designated P_2 comprises the nucleotide sequence encoding the amino acid sequence of the fourth variable and constant region (V4 and C4). In one embodiment of the present invention the forth variable region is surrounded by SacI and XhoI restriction enzyme sites.

35

The nucleotide sequence construct designated S_{1265gp120} comprises the nucleotide sequence encoding amino acid sequence of the fifth variable and constant region (V5 and C5). S_{1265gp120} further comprises a nucleotide sequence encoding a C-terminal stop codon.

- 5 The nucleotide sequence construct designated P_{4gp140} comprises the nucleotide sequence encoding amino acid sequence of the transmembrane spanning region. P_{4gp140} further comprises a nucleotide sequence encoding a C-terminal stop codon prior to the transmembrane spanning region.
- 10 The nucleotide sequence construct designated P_{4gp160} comprises the nucleotide sequence encoding amino acid sequence of the transmembrane spanning region (trans membrane spanning domain: TMD). In a preferred embodiment of the present invention the transmembrane spanning region is surrounded by HindIII and SacII restriction enzyme sites.
- 15 The term "sequence identity" indicates the degree of identity between two amino acid sequences or between two nucleotide sequences calculated by the Wilbur-Lipman alignment method (Wilbur et al, 1983).

- The nucleotide sequence constructs with the formula (I), (II), (III), or (IV) illustrates the flexibility in the present invention. By producing a gene with the described method enables the production of a plethora of antigens with various immunogenic epitopes and various advantages for production and vaccine purposes. To further illustrate the flexibility of the invention, other changes and mutations are suggested below.
- 20
 - 25 In order to improve the immunogenicity of the nucleotide sequence constructs of the invention it is suggested to change the nucleotide sequence such that one or more glycosylation sites are removed in the amino acid sequence. By removal of shielding glycosylations, epitopes are revealed to the immune system of the mammal rendering the construct more immunogenic. The increased immunogenicity can be determined by an improved virus neutralisation. Changes in the nucleotide sequence such that one or more N-linked glycosylation sites are removed in the amino acid sequence is well known by the person skilled in the art. Potential glycosylation sites are N in the amino acid sequences N-X-T or N-X-S (wherein X is any amino acid besides P). The glycosylation site can be removed by changing N to any amino acid, changing X to a P, or changing T to any amino acid. It is preferred that N is changed to Q by an A to C mutation at the first nucleotide in the codon, and a C to G mutation at the third nucleotide in the codon. This is preferred to increase the
 - 30
 - 35

GC content in the nucleotide sequence construct. As an alternative N is changed to Q by an A to C mutation at the first nucleotide in the codon, and a C to A mutation at the third nucleotide in the codon. Preferred mutations in the synthetic BX08 envelope gene to remove potential N-linked glycosylation sites in V1 and/or V2 are A307C + C309A and/or A325C + 5 C327G and/or A340C + C342A and/or A385C + C387A and/or A469C + C471A. Examples of such changes is illustrated in SEQ ID NOs: 47, 49, and 51.

For historical reasons the HIVs have been divided into syncytia inducing strains and non syncytia inducing strains. The assay to determine whether a strain is syncytia inducing is 10 described in Verrier et al 1997, hereby incorporated by reference. It is presently known, that viruses utilising the CXCR4 co-receptor are syncytia inducing strains. It is also, at the present, known that the binding site for the CXCR4 involves the third variable region (V3). In a preferred embodiment the nucleotide sequence construct is changed to create a binding site for the CXCR4 co-receptor. It is presently performed in the third variable regions, 15 preferably by the mutation G865C + A866G.

It is well established that the HIV envelope comprises immunodominant epitopes. An immunodominant epitope is an epitope that most antibodies from the mammal are directed against. The antibodies directed against these immunodominant epitopes may have little 20 effect in elimination of the virus. It is therefore anticipated that modification of the immunodominant epitopes will induce antibodies directed against other parts of the envelope leading to a better elimination and neutralisation of the virus. By modification is understood any change in the nucleotide sequence encoding an immunodominant epitope in the amino acid sequence such that said amino acid sequence no longer contains an immunodominant 25 epitope. Thus, modification includes removal of the immunodominant epitope and decrease of immunogenicity performed by mutagenesis. In a preferred embodiment of the present invention an immunodominant epitope in the third variable region (V3) is modified, such as deleted or altered. In a much preferred embodiment the nucleotides 793-897 are deleted. In yet another preferred embodiment of the present invention an immunodominant epitope 30 has been removed from gp41, such as deleted. This is performed in P₇ or P₈ by elimination of the nucleotides 1654-1710.

It is anticipated that when gp120 is dissociated from gp41 in a vaccine or antigen, two immunodominant epitopes, one on each protein, are exposed and antibodies are directed 35 against these in the mammal. In the infectious virus, gp120 is coiled on top of gp41 and the gp120/gp41 is most likely organised in a trimer, so that these immunodominant epitopes are

- hidden and therefore less elimination of virus is observed. By removing the cleavage site between gp41 and gp120 a full length gp160, gp150, or gp140 can be obtained with a covalent binding between gp41 and gp120. Removal of the cleavage site between gp41 and gp120 is preferably performed by a mutation at position C1423A. An example of such a 5 mutation is illustrated in the mutation of S_{1265Xhol} (SEQ ID NO: 17) to S_{1265gp160} (SEQ ID NO: 21).

- In order to stabilise the full length gp160, gp150, and gp140 for example when the cleavage site between gp41 and gp120 has been removed as described above, cysteins can be 10 inserted, preferably inside the gp41 helix creating disulphide bounds to stabilise a trimer of gp41s. In a preferred embodiment of the present invention the cysteins are inserted by the mutation 1618:CTCCAGGC:1625 to 1618:TGCTGCGG:1625. An example of such a change is illustrated in SEQ ID NO: 25.
- 15 The above mentioned decrease in immunodominant epitopes combined with the increase in immunogenicity of the other epitopes is expected to greatly enhance the efficacy of the nucleotide sequence construct as a vaccine.

- During the production of the nucleotide sequence construct, it is convenient to ligate the 20 snuts into pieces. The pieces, as described above, are characterised by their reversible assembly as there are no duplicate restriction enzyme sites. In a preferred embodiment one piece (herein designated P₃) contains P₁, S_{495ClaI}, S_{650-720EcoRI}, and P₂. Another piece (herein designated P₈) contains S_{1265Xhol}, S_{1465PstI}, and P_{4gp160}. Yet another piece (herein designated P₇) contains S_{1265Xhol}, S_{1465PstI}, P_{4gp160}, S_{2060SacII}, and P₅.
- 25 One advantage of the present nucleotide sequence construct is the easy access to exchange and alterations in the content and function of the nucleotide sequence and the encoded amino acid sequence. In one embodiment the nucleotide sequence coding for a functional region or parts thereof of the amino acid sequence is repeated. The repeat could 30 be back-to-back or a functional region or parts thereof could be repeated somewhere else in the sequence. Repeated could mean two (one repetition) but could also be three, six, or nine repeats. In a much preferred embodiment the repetition nucleotide sequence codes for amino acids in the third variable region.
- 35 In order to improve the protective capabilities of the invention against infections with HIV, one embodiment of the invention relates to the combination of epitopes. The present

nucleotide sequence construct allows insertion of one or more new nucleotide sequences isolated from another group and/or subtype of HIV and/or isolated from another patient. Hereby a vaccine or antigen with two or more epitopes from two or more HIVs is obtained. In a preferred embodiment, the V3 is replaced by the new nucleotide sequence. In a much 5 preferred embodiment, the new nucleotide sequence codes for amino acids in the third variable region of a different HIV isolate.

In order to improve the efficacy of the vaccine, aiming at raising cellular immunity, a nucleotide sequence coding for a T-helper cell epitope is included in the nucleotide 10 sequence construct. The nucleotide sequence coding for a T-helper cell epitope or a T-helper cell epitope containing amino acid sequence can be put in anywhere in the nucleotide sequence construct as long as it does not interact with the function of the envelope molecule. However, it is preferably placed in the tail of the nucleotide sequence construct or between the leader sequence and the envelope gene. The T-helper epitopes are preferably selected 15 from core proteins such as P24gag or from a non-HIV pathogen such as virus, bacteria, e.g. BCG antigen 85. For a therapeutic vaccine an HIV helper epitope is preferred since the patient is already primed by the HIV infection. For a prophylactic vaccine, a T-helper cell epitope from a frequently occurring non HIV pathogen such as Hepatitis B, BCG, CMV, EBV is preferred. Also, since the synthetic BX08 envelope genes may contain T-helper cell 20 epitopes in addition to important antibody epitopes, the synthetic BX08 vaccine genes can be mixed with other DNA vaccines to improve the efficacy of the other DNA vaccine.

One aspect of the present invention relates to individualised immunotherapy, wherein the virus from a newly diagnosed patient is directly cloned, the envelope or subunits 25 corresponding to snuts or pieces is produced with highly expressed codons, inserted into any of the nucleotide sequence constructs described above and administered to the patient as a vaccine. Hereby a therapeutic DNA vaccine is obtained, that will help the patient to break immunetolerance or induce/reinduce an appropriate immune response. In one embodiment the variable regions of the virus are produced with highly expressed codons and exchanged 30 into any of the nucleotide sequence constructs described above.

In one embodiment of the invention, the nucleotide sequence construct as described above satisfies at least one of the following criteria:

- a) serum extracted from a Macaque primate which has been immunised by administration of 35 an expression vector containing the nucleotide sequence construct is capable of eliminating SHIV as determined by quantitative PCR and/or virus culturing.

- b) serum extracted from a primate which has been immunised by administration of an expression vector containing the nucleotide sequence construct is capable of neutralising HIV-1 BX08 and /or other HIV-1 strains *in vitro*.
- c) serum, extracted from a mouse which has been immunised by administration of an expression vector containing the nucleotide sequence construct four times in intervals of three weeks and boosted after 15 weeks, is capable of decreasing the concentration of HIV-antigen in a culture of HIV, serum or PBMCs by at least 50%. An example of such procedure is shown in example 9.
- 10 In one embodiment of the invention, the nucleotide sequence construct of the invention, is used in medicine. That is, it is used as a vaccine, for the production of a recombinant protein, such that the recombinant protein is used as a vaccine, or the nucleotide sequence construct or the recombinant protein is used in a diagnostic composition.
Thus, the nucleotide sequence construct of the invention can be used for the manufacture of
15 a vaccine for the prophylactics of infection with HIV in humans.

Intramuscular inoculation of nucleotide constructs, i.e. DNA plasmids encoding proteins have been shown to result in the generation of the encoded protein *in situ* in muscle cells and dendritic cells. By using cDNA plasmids encoding viral proteins, both antibody and CTL
20 responses were generated, providing homologous and heterologous protection against subsequent challenge with either the homologous or cross-strain reaction, respectively. The standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA therapeutics of this invention. While standard techniques of molecular biology are therefore sufficient for the production of the products of this
25 invention, the specific constructs disclosed herein provide novel therapeutics which can produce cross-strain protection, a result heretofore unattainable with standard inactivated whole virus or subunit protein vaccines.

The amount of expressible DNA to be introduced to a vaccine recipient will depend on the strength of the transcription and translation promoters used in the DNA construct, and on the
30 immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of about 10 µg to 300 µg is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, inoculation by gene gun preferably DNA coated gold particles, and other modes of administration such as intraperitoneal, intravenous, peroral, topical, vaginal, rectal, intranasal
35 or by inhalation delivery are also contemplated. It is also contemplated that booster

vaccinations are to be provided. It is further contemplated that booster vaccinations with recombinant antigens are to be provided, administered as described above.

The DNA may be naked, that is, unassociated with any proteins, adjuvants or other agents
5 which impact on the recipients immune system. In this case, it is desirable for the DNA to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the DNA may be associated with surfactants, liposomes, such as lecithin liposomes or other liposomes, such as ISCOMs, known in the art, as a DNA-liposome mixture, (see for example WO93/24640) or the DNA may be associated with and
10 adjuvant known in the art to boost immune responses, such as a protein or other carrier.

Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, detergents, viral proteins and other transfection facilitating agents may also be used to advantage. These agents are generally referred to as transfection facilitating agents and as pharmaceutically acceptable carriers.

15

Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems may be used. A wide range of suitable mammalian cells are available from a wide range of sources (e.g. the American Type Culture Collection, Rockland, Dm; also, see e.g. Ausubel et al. 1992). The method of transformation or transfection and the
20 choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described e.g. in Ausubel et al 1992; expression vehicles may be chosen from those provided e.g. in P.H. Pouwels et al. 1985.

In one embodiment of the present invention the protein encoded by the nucleotide sequence
25 construct is produced by introduction into a suitable mammalian cell to create a stably-transfected mammalian cell line capable of producing the recombinant protein. A number of vectors suitable for stable transfection of mammalian cells are available to the public e.g. in *Cloning Vectors: A Laboratory manual* (P.H. Pouwels et al. 1985); methods for constructing such cell lines are also publicly available, e.g. in Ausubel et al. 1992.

30

Standard reference works describing the general principles of recombinant DNA technology include Watson, J.D. et al 1987; Darnell, J.E. et al 1986; Old, R.W. et al, 1981; Maniatis, T. et al 1989; and Ausubel et al.1992.

Figure legends

The invention is further illustrated in the following non-limiting examples and the drawing wherein

- 5 Figure 1 provides the codon preference of highly expressed proteins in human cells.

Figure 2 illustrates the outline of gp120, gp140, gp150, and gp160 encoding synthetic genes derived from the wild type sequence at the top. Variable (V) and constant (C) regions are shown together with the leader peptide (LP) and the transmembrane spanning domain (TMD). The approximate nucleotide positions of the restriction enzyme sites are shown. 10 The approximate position of the three restriction enzyme sites dividing the full-length gp160 gene into the three pieces each containing only unique restriction enzyme sites are shown in bold.

15 Figure 3 building of the synthetic gp120 gene. Variable (V) and constant (C) regions are shown together with the leader peptide (LP) and the transmembrane spanning domain (TMD). The approximate nucleotide positions of the restriction enzyme sites are shown.

Figure 4 building of the synthetic gp140 gene. Variable (V) and constant (C) regions are shown together with the leader peptide (LP) and the transmembrane spanning domain (TMD). The approximate nucleotide positions of the restriction enzyme sites are shown. 20

Figure 5 building of the synthetic gp150 gene. Variable (V) and constant (C) regions are shown together with the leader peptide (LP) and the transmembrane spanning domain (TMD). The approximate nucleotide positions of the restriction enzyme sites are shown. 25

Figure 6 building of the synthetic gp160 gene. Variable (V) and constant (C) regions are shown together with the leader peptide (LP) and the transmembrane spanning domain (TMD). The approximate nucleotide positions of the restriction enzyme sites are shown. 30

Figure 7 illustrates the codons coding amino acids in general

Figure 8 illustrates how overlapping PCR is performed.

35 Figure 9 illustrates how PCR using conserved flanking ends is performed.

Figure 10 illustrates how $S_{1265Xhol}$ is produced using complementary strands (minigene-approach) technology. The $S_{1265Xhol}$ is ligated from three sets of complementary strands into the vector pBluescript KS⁺ between restriction enzyme sites *Xhol* and *PstI*.

5

Figure 11 illustrates how $S_{1465PstI}$ is produced. The same approach, as the approach used for the production of $S_{1265Xhol}$, was used except that only two sets of complementary strands were used.

- 10 Figure 12 illustrates the assembly of P_1 . The $S_{O-N-Lang}$ and $S_{235EcoRV}$ are ligated into the *XbaI* and *PstI* site of the $S_{375PstI}$ containing plasmid.

Figure 13 illustrates the assembly of P_2 . The $S_{900XbaI}$ was excised by *HindIII* and *SacI* from its plasmid and ligated with $S_{990SacI}$ (*SacI-Spel*) into the $S_{110Spal}$ plasmid that was opened at the *HindIII* and *Spel* sites.

15

Figure 14 illustrates the assembly of P_3 . $S_{495Cial}$ (*ClaI-EcoRI*) and $S_{650-720EcoRI}$ (*EcoRI-XbaI*) and P_2 (*XbaI-Xhol*) were ligated simultaneously into the P_1 plasmid opened at the *ClaI* and *Xhol* sites to obtain the P_3 plasmid.

20

Figure 15 illustrates the assembly of P_{4gp160} . $S_{1890HindIII}$ (*SacI-HindIII*) and $S_{1700EagI}$ (*HindIII-EagI*) were ligated simultaneously into the $S_{1630XbaI}$ plasmid opened by *SacII* and *EagI*.

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Figure 16 illustrates the assembly of P_5 . $S_{2190Cial}$ (*ClaI-PstI*) and $S_{2330PstI}$ (*PstI-EcoRI*) were ligated into the $S_{2425EcoRI}$ plasmid opened by *ClaI* and *EcoRI*.

Figure 17 illustrates the assembly of P_{8gp160} . $S_{1465PstI}$ (*XbaI-PstI*) and $S_{1265Xhol}$ (*PstI-Xhol*) were ligated into the P_{4gp160} plasmid opened by *XbaI* and *Xhol*.

30

Figure 18 illustrates the assembly of P_{8gp160} . $S_{1465PstI}$ (*XbaI-PstI*) and $S_{1265Xhol}$ (*PstI-Xhol*) were ligated into the plasmid containing P_{4gp150} with the stop codon. P_{4gp150} plasmid was opened at the *XbaI* and *Xhol* sites for the ligation.

35

Figure 19 illustrates the assembly of P_{8gp140} . $S_{1465PstI}$ (*XbaI-PstI*) and $S_{1265Xhol}$ (*PstI-Xhol*) were ligated into the plasmid containing P_{4gp140} with a stop codon. P_{4gp140} plasmid was opened at the *XbaI* and *Xhol* sites for the ligation.

Figure 20 illustrates the assembly of P_{8gp41}. Two complementary nucleotide strands 1265gp41S and 1265gp41AS designed with overhang creating a 5' Xhol and a 3' PstI restriction enzyme site were annealed and ligated into the piece 8 which is already opened at the Xhol and PstI sites whereby S₁₂₆₅ is deleted.

Figure 21 illustrates the assembly of P₇. P₈ (Xhol-SacII) and S_{2060SacII} (SacII-ClaI) were ligated into P₅ plasmid opened at Xhol and C/IaI.

- 10 Figure 22a SDS PAGE of ³⁵S-labelled HIV-1 BX08 envelope glycoproteins radio-immuno precipitated from transiently transfected 293 cells using the indicated plasmids. Cell pellet (membrane bound antigens) or cell supernatant (secreted antigens) were precipitated by a polyclonal anti-HIV-1 antibody pool. Lane 1: untransfected cells. Lane 2: supernatant from syn.gp120_{MN} transfected cells. Lane 3: cell pellet from wt.gp160_{BX08} transfected cells.
- 15 Lane 4: cell pellet from cells co-transfected by wt.gp160_{BX08} and pRev. Lane 5: Mwt. marker. Lane 6: cell pellet from syn.gp160_{BX08} transfected 293 cells. Lane 7: cell pellet from syn.gp150_{BX08} transfected 293 cells. Lane 8: supernatant from syn.gp140_{BX08} transfected cells. Lane 9: supernatant from syn.gp120_{BX08} transfected cells.
- 20 Figure 22b is an SDS-PAGE of ³⁵S-labeled HIV-1 BX08 envelope glycoproteins radio-immune precipitated from transiently transfected 293 cells as cell pellet (membrane bound) or cell supernatant (secreted antigens) by anti-HIV-1 antibody pool using the indicated plasmids. Lane 1: untransfected 293 cells. Lane 2: cell pellet from syn.gp160MN transfected 293 cells as positive control (Vinner et al 1999). Lane 3: Cell supernatant from 25 syn.gp120MN transfected 293 cells as positive control (Vinner et al 1999). Lane 4: Cell supernatant from syn.gp120BX08 transfected 293 cells demonstrating a glycoprotein band of 120 kDa. Lane 5: Cell supernatant from syn.gp140BX08 transfected 293 cells demonstrating a glycoprotein band of 120 kDa. Lane 6: Mwt. marker. Lane 7 at two different exposure times: Cell pellet from syn.gp150BX08 transfected 293 cells 30 demonstrating a glycoprotein band of 120 kDa (lower gp30 band is not well seen in this exposure). Lane 8: Cell supernatant from syn.gp150BX08 transfected 293 cells showing no secreted proteins (all protein is membrane bound, see lane 7).

Figure 22c show fluorescent microscopy of U87.CD4.CCR5 cells transfected with BX08

- 35 gp160 genes plus pGFP. Panel A: cells transfected with empty WRG7079 vector plus pGFP showing no syncytia. Panel B: cells transfected with wild type BX08gp160 gene

plus pGFP showing some syncytia. Panel C: cells transfected with synBX08gp160 plus pGFP showing extreme degree of syncytia formation. This demonstrates expression, functionality, and tropism of the expressed BX08 glycoprotein with much more expressed functionally active gp160 from the synthetic BX08 gene.

5

Figure 23 shows the anti-Env-V3 BX08 antibody titers (IgG1). Panels show individual mice DNA immunized with syn(gp140)BX08 plasmid either i.m. (left panel) or by gene gun (right panel), respectively. Immunization time points are indicated by arrows.

- 10 Figure 24 shows a Western Blotting of (from left to right) one control strip, followed by sera (1:50) from 2 mice i.m. immunized with synBX08gp120, 2 mice i.m. immunized with synBX08gp140, 2 mice i.m. immunized with synBX08gp150, and 2 mice immunized with synBX08gp160, followed by 2 mice gene gun immunized with synBX08gp120, 2 mice gene gun immunized with synBX08gp140, 2 mice gene gun immunized with syn
- 15 BX08gp150, and 2 mice gene gun immunized with synBX08gp160 respectively. Strip 5 is a mouse 5.1 DNA immunized i.m. with synBX08gp140 plasmid (same mouse as in figure 23). Plasma was examined at week 18. The position of gp160 (spiked with four coupled gp51), gp120 and gp41 is indicated at the right. A positive reaction to HIV glycoproteins further demonstrates the mouse anti-HIV immunoglobulin reacting to HIV of a strain (IIIB)
- 20 different from BX08 to illustrate cross-strain reactivity.

Figure 25 Theoretical example of calculation of the 50% inhibitory concentration (IC_{50}) values. IC_{50} for each mouse serum is determined by interpolation from the plots of percent inhibition versus the dilution of serum.

25

- Figure 26 CTL responses were measured at week 18 to the mouse H-2D^d restricted BX08 V3 CTL epitope (IGPGGRAFYTT) for BALB/c mice (H-2D^d) i.m. immunized at week 0, 9, and 15 with the synthetic vaccine genes: syn(gp120)_{BX08}, syn(gp140)_{BX08}, syn(gp150)_{BX08}, and syn(gp160)_{BX08}, respectively, and median values of different E:T ratios for groups of
- 30 mice are shown (26A). Intramuscular DNA immunization with syn(gp150)_{BX08} induced a higher CTL response when injected i.m. in high amounts versus gene gun inoculation of skin (26B).

- Figure 27 Summary of western immuno blotting assay of mice sera (1:40) collected at week 35 0, 9, and 18 from mice genetically immunized with syn(gp120)_{BX08}, syn(gp140)_{BX08},

syn(gp150_{BX08}), syn(gp160_{BX08}), wt(gp160_{BX08}), and wt(gp160_{BX08}) plus pRev, respectively.
Percent responders in groups of 17-25 mice against gp120 and gp41 are shown.

Figure 28 IgG anti-rgp120 (IIIB) antibody titers of individual mice inoculated at week 0, 9, 15
5 (28A), or gene gun immunized at week 3, 6, 9, and 15 (28B) with the syn(gp150_{BX08}) DNA
vaccine.

Figure 29 IgG antibody titers to HIV-1 rgp120_{IIIB}. Median titers are shown from groups of
mice i.m. inoculated at week 0, 9, and 15 (29A), or gene gun immunized at week 0, 3, 6,
10 9, and 15 (29B) with the synthetic genes syn(gp120_{BX08}), syn(gp140_{BX08}), syn(gp150_{BX08}),
and syn(gp160_{BX08}), respectively.

Examples

Example 1: Designing the nucleotide sequence construct

Initially the overall layout of the nucleotide sequence construct is decided. The overall layout comprises the various derivatives the gene will be expressed as. For BX08 these include, but

5 are not restricted to gp160, gp150, gp140, gp120, and gp41.

Next, the vehicle of expression (plasmid or virus) is to be determined: Preparation for a suitable vector determines both need for leader sequence, terminal restriction enzyme sites and whether or not an N- or C-terminal protein tag is to be considered (Poly-his, Myc- antibody-epitop, etc.). For BX08 a plasmid expression vehicle was chosen. All native wild

10 type HIV codons are systematically exchanged with the codons most frequently represented in a pool of highly expressed human genes (figure 1). By this exchange the amino acid sequence is conserved while the nucleotide sequence is dramatically altered. Thus, gene structures like overlapping reading frames (e.g. *vpu*, *rev*, and *tat*) or secondary structures (e.g. RRE) are most likely destroyed whereas protein cleavage sites, and glycosylation sites

15 are maintained. The 100% amino acid identity between wtBX08 and synthetic BX08 in the present examples should be calculated after the initial Ala-Ser amino acid sequence, as that sequence is a part of the 6 amino acid sequence long *Nhe*I restriction enzyme site.

Depending on the restriction enzyme sites located in the expression vector it is decided

20 which restriction enzyme sites can be present (tolerated) throughout the finished gene construct. The terminal restriction enzyme sites of the synthetic gene must remain unique to enable cloning into the vector chosen for expression. General requirements for restriction enzyme sites of choice: Preferably creating cohesive ends facilitating ligation, creating no compatible ends with adjacent restriction enzyme sites (e.g. *Bam*H/I/*Bgl*II), and being efficient
25 cutters. For BX08 the restriction enzyme sites accepted were the ones present in the polylinker of the pBluescript cloning vectors (*Eag*I, *Mlu*I, *Eco*RV, *Pst*I, *Cla*I, *Eco*RI, *Xba*I, *Sac*I, *Spe*I, *Xho*I, *Hind*III, *Sac*II, *Not*I, *Bam*H/I, *Sma*I, *Sal*I, *Dra*I, *Kpn*I with the exception of *Bgl*II and *Nhe*I). This was decided to satisfy the original cloning strategy using individual cloning of snuts in pBluescript with restriction enzyme cleaved (trimmed) ends after PCR
30 amplification, which is not necessary when blunt-end cloning and assembling of complementary oligonucleotides are employed. All locations at which the selected restriction enzyme sites can be introduced by silent mutations (keeping 100% loyal to the amino acid sequence) are identified using the SILMUT software or equivalent.

- From these possible restriction enzyme sites, a selection of restriction enzyme sites are introduced by silent nucleotide substitutions around functional regions of choice of the corresponding gene (e.g. RRE) or gene products (e.g. variable region 1 (V1), V2, V3, CD4 binding area, transmembrane domain, and regions of immunological significans, etc.).
- 5 Restriction enzyme sites are located at terminal positions of subcloned snuts (building entities) but additional restriction enzyme sites may be present within subunits. For BX08 the construct was initially to be cloned in the WRG7079 vector containing a tPA-leader sequence. Cloning sites were 5'-*Nhe*I → *Bam*H-I-3'. The entire humanised BX08 sequence was divided into thirds: 5'-*Nhe*I → *Xho*I → *Sac*II → *Bam*H-I-3'. These sites were chosen in
- 10 this particular order because it resembles the polylinker of pBluescript (KS') enabling successive ligations of the assembled thirds in this cloning vector. Within these thirds restriction enzyme sites were kept unique. Next, restriction enzyme sites were placed to flank the functional regions chosen as follows:
- A. (5'-V1): *Eco*RV-235: Between C1 and V1. Alternatives: 3×*Hind*III (already excluded)
- 15 because exclusive use at position 1890) or *Eco*RV.
- B. (V1-3'): Only alternative *Pst*I 375.
- C. (5'-V2): as B.
- D. (V2-3'): Alternatives: *Spel*, *Cla*I 495. *Cla*I chosen because it is closer to V2.
- E. *Eco*RI 650 placed because next possible site was too far away.
- 20 F. (5'-V3): *Bgl*II 720 was the alternative closest to the V3 region and further more unique.
- G. (V3-3'): Alternatives *Xho*I (excluded) and *Xba*I 900 located very close to the V3 loop.
- H. (5'-V4) *Sac*I 990: alternatively *Eco*RI or *Bam*H-I (both excluded)
- I. (V4-3'): Alternatives *Spel* 1110, *Kpn*I 1145, *Pst*I 1135. *Pst*I already used, *Spel* chosen because of distance to previous site (*Sac*I 990).
- 25 J. (5'-V5): *Xho*I initially determined.
- K. (Fusion peptide-3') *Pst*I 1465 was the closest alternative to *Xho*I 1265.
- L. (5'-Immunodominant region): *Xba*I 1630 chosen among *Eco*RV (blunt end), *Pst*I and *Xho*I (both already used).
- M. (Immunodominant region-3'): *Eag*I 1700 perfect location.
- 30 N. (C34 and C43 -3' (Chan, Fass, et al. 1997), and 5'-trans membrane domain): *Sac*II. No alternatives.
- O. (Trans membrane domain -3'): *Sac*II 2060 already present.
- P. *Cla*I 2190 perfect position in relation to previous RE-site.
- Q: *Pst*I perfect position in relation to previous RE-site.
- 35 R. *Eco*RI 2400 introduced to facilitate later substitution of terminal snut.
- S. *Bam*H-I 2454 determined by the WRG7079 vector.

Remove undesired restriction enzyme sites by nucleotide substitutions (keeping loyal to the amino acid sequence). Nucleotide substitution should preferably create codon frequently used in highly expressed human genes (figure 1). If that is not possible, the codons should

5 be the selected from the regular codons (figure 7). The substitutions made to the second nucleotide sequence to obtain desired restriction enzyme sites are shown in Table 2.

Table 2 lists silent nucleotide substitutions in the humanised BX08 envelope sequence. Substitutions were made to create or delete restriction enzyme sites.

Position:	substitution	Remarks:
138	c → g	creates Mlu I site on pos. 134-139
240	c → t	creates EcoRV site on pos. 238-243
501	c → a	creates Cla I site on pos. 501-506
502	a → t	do
503	g → c	do
504	c → g	do
657	c → a	creates EcoRI site on pos. 656-661
660	c → t	do
724	c → a	creates Bgl II site on pos. 724-729
726	c → g	do
727	a → t	do
728	g → c	do
729	c → t	do
840	c → t	EagI site is eliminated
904	a → t	creates Xba I site on pos. 904-909
905	g → c	do
906	c → t	do
907	c → a	do
909	c → a	do
994	a → t	creates Sac I site on pos. 990-995
995	g → c	do
1116	c → t	creates Spel site on pos. 1114-1119
1119	c → t	do
1273	a → t	creates Xhol site on pos. 1272-1277
1274	g → c	do
1275	c → g	do
1293	c → t	Bgl II site is eliminated
1443	c → t	BstXI site is eliminated
1452	g → c	do
1467	c → t	PstI site on pos. 1466-1471
1470	c → a	do
1590	g → c	PstI site on pos. 1588-1593 is eliminated
1620	g → c	PstI site on pos. 1618-1623 is eliminated
1638	c → t	creates XbaI site on pos. 1638-1643
1641	g → a	do
1653	g → c	PstI site is eliminated
1687	a → t	PstI site is eliminated
1688	g → c	PstI site is eliminated
1710	c → g	creates EagI site on pos. 1709-1714
1758	c → t	Bgl II site is eliminated
1875	g → c	PstI site is eliminated
1893	c → a	Hind III on pos. 1893-1898
1897	c → t	do
1944	c → t	Bgl II site is eliminated

Position:	substitution	Remarks:
2199	c → t	Cla I site on pos. 2198-2203
2202	c → t	do
2203	c → t	do
2253	c → g	SacII site is eliminated
2292	g → c	PstI site is eliminated
2320	a → t	PstI site on pos. 2321-2326 is eliminated
2321	a → t	do
2322	g → t	do
2325	c → a	do
2430	c → a	creates EcoRI site on pos. 2429-2434
2433	c → t	do

Example 2: synthesis of oligos

In order to clone the individual snuts, nucleotide strands were synthesised or purchased. In total 28 synthetic nucleotide strands were synthesised. Nucleotide strands were synthesised

- 5 by standard 0.2 µmol β-cyanoethyl-phosphoramidite chemistry on an Applied Biosystems DNA synthesiser model 392, employing 2000 Å CPG columns (Cruachem, Glasgow, Scotland), acetonitrile containing less than 0.001% water (Labscan, Dublin, Ireland) and standard DNA-synthesis chemicals from Cruachem, including phosphoramidites at 0.1 M and Tetrahydrofuran/N-methylimidazole as cap B solution. The nucleotide strands O-N-C
- 10 and 119MS-RC (for cloning of snut O-N-Lang), 650-E-BG and 720-XBAC-31 (for cloning of snut 650-720-EcoRI), 2425esup and 2425ESdo (for cloning of snut 2425-E-S) were synthesised with 5' end "trityl on" and purified on "Oligonucleotide Purification Cartridges" (Perkin Elmer, CA, USA) as described by the manufacturer. Other nucleotide strands (235-ECO5, 375-pst1.seq, 495-Cla1.seq, 900-XbaI, 990-sac1, 1110-SPE, 1630-Xba.seq, 1700-
- 15 Eag.seq, 17-Eag.seq, 1890-Hind.MPD, 2060-sac, 2190-cla, 2330-pst) were synthesised with 5' end "trityl off" and purified by standard ethanol precipitation. Oligoies 1265-1UP, 1265-1DO, 1265-2UP, 1265-2DO, 1265-3UP, 1265-3DO, 1465-1UP, 1465-1DO, 1465-2UP, 1465-2DO were purchased from Pharmacia.

Example 3: Cloning of snuts

- 20 The nucleotide sequence construct was designed in 17 DNA small pieces called snuts (Table 3) encompassing important structures like variable and constant regions each flanked with restriction enzyme (RE) sites to facilitate cassette exchange within each third of the gene: *Nhel-Xhol*, *Xhol-SacII*, *SacII-BamHI*.
- 25 Each snut was cloned individually in a commercial vector (pBluescriptKS or pMOSblue) and kept as individual DNA plasmids, named after the snut which gives the nucleotide position of the RE in the BX08.

Table 3 list the snuts by their name and cloning vector.

Name	Cloning vector:
S _{O-N-Lang}	pMOSblue
S _{235EcoRV}	pMOSblue
S _{375PstI}	pBluescriptSK
S _{495ClaI}	pMOSblue
S _{650-720EcoRI}	pMOSblue
S _{900XbaI}	pMOSblue
S _{990SacI}	pMOSblue
S _{1110SphI}	pMOSblue
S _{1265Xhol}	pBluescriptSK
S _{1465PstI}	pBluescriptSK
S _{1630XbaI}	pBluescriptSK
S _{1700EagI}	pBluescriptSK
S _{1890HindIII}	pBluescriptSK
S _{2060SacII}	pMOSblue
S _{2190ClaI}	pMOSblue
S _{2330PstI}	pMOSblue
S _{2425ES}	pBluescriptSK

Three principally different methods were used to obtain the dsDNA corresponding to each of
 5 the 17 snuts needed to build the synthetic BX08 genes.

1) "Overlapping" PCR: is based on the use of two ssDNA template nucleotide strands (forward and reverse) that complement each other in their 3'-end (figure 8). During the first PCR cycle, both templates annealed to each other at the 3'-ends allowing the full-length 10 polymerisation of each complementary strand during the elongation step. The newly polymerized dsDNA strand are then amplified during the following cycles using an adequate forward and reverse primers set (figure 8).

Snut O-N-LANG: (S_{O-N-Lang}) two ng of the forward template nucleotide strands O-N-C and 2 15 ng of the reward template-nucleotide strand 119MS-RC were mixed together with 50pmoles of the forward primer O-N-LANG-5 (5'-CTAGCTA-GCGCGGCCGACCGCCT -3') and 50pmoles of the reverse primer O-N-LANG-3 (5'-CTCGATATCCTCGTGCATCTGCTC -3') in a 100µl PCR reaction volume containing 0.2mM dNTP's, 1x ExpandHF buffer with MgCl₂ (1.5mM) and 2.6 units of enzyme mix (Expand™ High fidelity PCR system from Boehringer 20 Mannheim). The PCR was performed with the PE Amp 9600 thermocycler (Perkin Elmer) using the following cycle conditions: initial denaturation at 94°C for 30 sec., followed by 30 cycles of 94°C for 15 sec., 65°C for 30 sec., 72°C for 45 sec., with a final elongation at 72°C for 5 min., and cooling to 4°C.

Snut 650-720-EcoRI: ($S_{650-720EcoRI}$) PCR amplification was performed as described for snut O-N-LANG. One μ g of the forward ssDNA template-oligonucleotide 650-E-BG and 1 μ g of the reverse ssDNA template-oligonucleotide 720-XBAC were mixed with 40pmoles of the forward primer 650-E-5 (5'-CCGGAATT-CGCCCCGTGGTGGCA-3') and 40 pmoles of the 5 reverse primer 720-X-3 (5'-CTGCTCTAGAGATGTTGCAGTGGGCCT-3').

2) "Normal" PCR amplification: Eleven nucleotide strands: 235-ECO5, 375-pst1, 900-xba1, 990-sac1, 1110-SPE, 1630-XBA, 1700-EAG, 1890-HIN, 2060-sac, 2190-cla, and 2330-pst, were designed with common 5' and 3' flanking sequences which allowed PCR amplification 10 with the same primer set (Forward primer : BX08-5 (5'-AGCGGATAACAATTCACACAGGA-3') and revers primer : BX08-3 (5'-CGCCAGGGTTTCCCAGTCACGAC-3') (Figure 9). The 495-Cla1 oligonucleotide was designed without a common flanking sequence and was therefore amplified with a specific set of primers 495-5N/495-3N (5'-GAATCGAT-CATCACCCAG-3' and 5'-GACGAATTCCGTGGGTGCACT-3'). Each oligonucleotide was 15 resuspended in 1ml of water and kept as a stock solution (approximatively 0.2 mM). PCR amplification was performed with the Expand™ High Fidelity PCR System from Boehringer Mannheim (Cat. No. 1759078). Four concentrations of template nucleotide strand were systematically used: undiluted stock solution, stock solution 10^{-1} , stock solution 10^{-2} , stock solution 10^{-3} . One to 5 μ l of synthetic ssDNA template was amplified using the following 20 conditions: BX08-5 (0.5 μ M), BX08-3 (0.5 μ M), 4 dNTP's (0.2mM), 1x ExpandHF buffer with MgCl₂ (1.5mM) and 2.6 units of enzyme mix. The PCR was performed using the PE Amp 9600 thermocycler (Perkin Elmer) using the following cycle conditions: initial denaturation at 94°C for 15 sec., followed by 30 cycles of 94°C for 15 sec., 65°C for 30 sec. and 72°C for 45 sec., with a final elongation at 72°C for 7 min., and cooling to 4°C.

25

3) Minigene approach : This method was used to synthesise $S_{1265Xhol}$, $S_{1465XbaI}$ and S_{2425ES} .

Snut 2425-E-S (S_{2425ES}): 100 picomoles of each oligonucleotide 2425ES-up (35-mer ; 5'-AATTGCCAGGGCTTCGAGCGCGCCCTGCTGTAAG-3') and 2425ES-do (35-mer ; GATCCTTACAGCAGGGCGCGCTCGAACG-CCTGGCG-3') were mixed together in a 100 μ l 30 final volume of annealing buffer containing NaCl 25mM , Tris 10mM and 1mM EDTA. After denaturation at 94°C for 15 min., the mixed oligonucleotides were allowed to anneal at 65°C during 15min.. The annealing temparature was allowed to slowly decrease from the 65°C to room temperature (22°C) during overnight incubation. The resulting double-strand dsDNA fragments harbored EcoRI- and BamHI-restriction sites overhangs that allowed direct cloning 35 in pBluescript KS(+) vector using standard cloning techniques (Maniatis 1996).

Snut 1265-Xhol (S_{1265Xhol}): This snut was built according to the strategy depicted in figure 10. Three minigenes were constructed following the same method described for snut 2425-E-S. These minigenes are named 1265-1, 1265-2 and 1265-3. The minigene 1265-1 results from the annealing of the oligonucleotides 1265-1up (68-mer ; 5'-TCG AGC AGC GGC AAG GAG ATT 5 TTC CGC CCC GGC GGC GAC ATGC GCG ACA ACT GGC GCA GCG AGC T-3') and 1265-1do (68-mer ; 5'-GTA CAG CTC GCT GCG CCA GTT GTC GCG CAT GTC GCC GCC GGG GCG G AAA ATC TCC TTG CCG CTG C-3'). 1265-2 results from the annealing of 1265-2up (61-mer ; 5'-GTA CAA GTA CAA GGT GGT GAA GAT CGA GCC CCT GGG CAT CGC CCC CAC CAA GGC CAA GCG C-3') and 1265-2do (63-mer ; 5'-CAC GCG GCG CTT GGC CTT GGT GGG GGC GAT GCC CAG GGG CTC GAT 10 CTT CAC CAC CTT GTA CTT-3'). Finally, 1265-3 results from the annealing of 1265-3up (69-mer ; 5'-CGC GTG GTG CAG CGC GAG AAG CGC GCC GTG GGC ATC GGC GCT ATG TTC CTC GGC TTC CTG GGC GCT GCA-3') and 1265-3do (59-mer ; 5'-GCG CCC AGG AAG CCG AGG AAC ATA GCG CCG ATG CCC ACG GCG CGC TTC TCG CGC TGC AC-3'). Each minigene were designed in order to present single strand overhangs at their 5' and 3'- ends that allow easy ligation and Xhol-PstI 15 direct cloning into pBlueScript KS+ vector.

Snut 1465-PstI (S_{1465PstI}): Two minigenes were constructed following the same method described for snut 2425-E-S. These minigenes are named 1465-1 and 1465-2. The minigene 1465-1 was obtained after annealing of 1465-1up (90-mer : 5'-GGC AGC ACC ATG GGC GCC 20 GCC AGC CTG ACC CTG ACC GTG CAG GCC CGC CAG CTG CTG AGC GGC ATC GTG CAG CAG CAG AAC AAC CTG CTG-3') and 1465-1do (98-mer : 5'-CGC GCA GCA GGT TGT TCT GCT GCT GCA CGA TGC CGC TCA GCA GCT GGC GGG CCT GCA CGG TCA GGG TCA GGC TGG CGG CGC CCA TGG TGC TGC CTG CA-3'), whereas minigene 1465-2 results from the annealing of 1465-2up (78-mer ; 5'-CGC GCC ATC GAG GCC CAG CAC CTG CTC CAG CTGA CCG TGT GGG GCA TCA AGC AGC TCC 25 AGG CCC GCG TGC TGG CT-3') and 1465-2do (78-mer ; 5'-CTA GAG CCA GCA CGC GGG CCT GGA GCT GCT TGA TGC CCC ACA CGG TCA GCT GGA GCA GGT GCT GCT GGG CCT CGA TGG-3'). Each minigene were designed in order to present single strand overhangs at their 5' and 3'- ends that allow easy ligation and PstI-XbaI direct cloning into pBlueScript KS+ vector using standard cloning techniques (Maniatis) (see figure 11).

30 Example 4: assembly of snuts to pieces.

The snut genes were then assembled into pieces (Table 4) so that unique restriction enzyme sites or mutagenesis can be used within each of these. This strategy will require fewer assemblies for optimal use of the cassette system. The following piece clones were made and kept individually for construction of the synBX08 gp160 gene (Figure 6):

Table 4 lists pieces by their name and their snut composition.

Piece name	snut composition	vector
P ₁	S _{O-N-LANG} -S _{235EcoRV} -S _{375PstI}	pBluescriptSK
P ₂	S _{900XbaI} -S _{990SacI} -S _{1110SpeI}	pMOSblue
P ₃	P ₁ -S _{495ClaI} -S _{650-720EcoRI} -P ₂	pBluescriptSK
P _{4gp160}	S _{1630XbaI} -S _{1700EagI} -S _{1890HinIII}	pBluescriptSK
P ₅	S _{2190ClaI} -S _{2330PstI} -S _{2425ES}	pBluescriptKS
P ₇	P _{8gp160} -S _{2060SacI} -P ₅	pBluescriptKS
P _{8gp160}	S _{1265Xhol} -S _{1465PstI} -P _{4gp160}	pBluescriptKS

Piece 1: The building strategy is shown in figure 12.

Preparation of the insert DNA: Five to 15µg of each plasmid O-N-LANG-cl7 and 235-EcoRV-cl5N, respectively, were double-digested by XbaI/EcoRV, and PstI/EcoRV, according to classical RE digestion procedure (Maniatis). The RE digestion products, were agarose gel purified according classical method (Maniatis). All RE digests were loaded on a 3% Nusieve 3:1(FMC), TBE 0.5X agarose gel and submitted to electrophoresis (7 Volts/mm during 2-3hours) until optimal fragment separation. The agarose-band containing the DNA fragments that correspond to the snut's sequence sizes (243-bp for O-N-LANG and 143-bp for 235-EcoRV) were excised from the gel. The DNA was extracted from agarose by centrifugation 20min at 5000g using a spin-X column (Costar cat#8160). Preparation of the vector: The snut 375-PstI klon1 was used as plasmid vector. Five µg were digested with XbaI and PstI. Removal of the polylinker XbaI/PstI fragment was performed by classical agarose gel purification, using a 0.9% Seakem-GTG agarose, TBE 0.5X gel. The linearised plasmid DNA was extracted from the agarose by filtration through spin-X column. All purified DNA fragments were quantified by spectrophotometry. Ligation: All three DNA fragments O-N-LANG (XbaI/EcoRV), 235-EcoRV (PstI/EcoRV) and 375-PstI(XbaI/PstI), were ligated together by classical ligation procedure, using an equimolar (vector:insert1:insert2) ratio of 1:1:1. Thus for, 200 ng (0.1 pmole) of XbaI/PstI-linearised 375-PstI-cl1 were mixed with 16 ng of O-N-LANG (XbaI/EcoRV) and 10 ng of 235-EcoRV (PstI/EcoRV) in a final reaction volume of 20µl of 1X ligation buffer containing 10U of T4 DNA ligase (Biolabs, cat#202S). The ligation was allowed overnight at 16°C. Transformation: Competent XL1-Blue bacteria (Stratagene cat#200130, transformation efficiency > 5•10⁶ col/µg) were transformed by classical heat-chock procedure : 1/10th of the pre-chilled ligation reaction was mixed with 50µl of competent bacteria. The mixture was allowed to stand in ice during 30 min. Bacteria were heat-shocked at 42°C during 45 sec. and then left 2 min. on ice before being resuspended in 450 µl of SOC medium. Transformed bacteria were incubated 1 hour at 37°C under shaking (250rpm) and plated on LB-ampicilin agar plates. The recombinant clones

- were allowed to grow 16 hours at 37°C. Colony screening: 10 to 50 recombinant colonies were screened by direct PCR screening according to the protocole described into the pMOSBlue blunt-ended cloning kit booklet (RPN 5110, Amersham). Each colony was picked and resuspended in 50µl of water. DNA was freed by a boilling procedure (100°C, 5 min). Ten µl of bacterial lysate were mixed to 1 µl of a 10mM solution of premixed 4 dNTP's , 1 µl of M13reverse primer (5pmoles/µl, 5'-CAGGAAACAGCTATGAC-3'), 1 µl of T7 primer (5pmoles/µl, 5'-TAATACGACTCACTATAGGG-3'), 5µl of 10x Expand HF buffer 2 (Boehringer Mannheim, cat#1759078), 0.5µl of Enzyme mix (Boehringer Mannheim, 5U/µl) in a final volume of 50µl. DNA amplification was performed with a thermo-cycler PE9600 (Perkin-Elmer) using the following cycling parameters: 94°C, 2min, 35 cycles(94°C, 30sec; 50°C, 15sec; 72°C,30sec); 72°C, 5min; 4°C hold. Five µl of the PCR products were analysed after electrophoresis on a 0.9% SeakemGTG , 0.5xTBE agarose gel. Nucleotide sequence confirmation: ds-DNA was purified from minicultures of the selected clones with the JETstar mini plasmid purification system (Genomed Inc.). Sequencing was performed using M13reverse and T7 primers and with the Big DyeTM Terminator Cycle Sequencing Ready reaction kit (Perkin-Elmer, Norwalk, Connecticut, P/N4303152) and the ABI-377 automated DNA sequenator (Applied Biosystems, Perkin-Elmer,Norwalk, Connecticut). Data were processed with the Sequence Navigator and Autoassembler softwares (Applied Biosystems, Perkin-Elmer,Norwalk, Connecticut).
- 20 **Piece 2:** The strategy for building that piece is depicted in figure 13. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described above for piece1, except the following:
- The linearised plasmid 1110-Spel-cl24M1 was used as vector after being digested by HindIII and Spel, and agarose gel purified.
 - A 166-bp HindIII/SacI, obtained from snut 900-XbaI-cl15, as well as a 130-bp SacI/Spel fragment, obtained from snut 990-SacI-cl14, were agarose gel purified.
 - Equimolar amount (0.1 pmoles) of the three DNA fragments described above were ligated in an one step ligation.
- 25 30 - 100µl of competent SCS110 bacteria (Stratagene cat# 200247) were transformed with 1/10th of the ligation products according to the manufactor instruction.
- Direct colony PCR screening was performed using T7 primer and pMOS-R (5'-GTTGTAAAACGACGCCAG-3').

Piece 3: The strategy for building that piece is depicted in figure 14. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described above for piece1, except the following:

- The plasmid piece1-cl33 was linearised by Clal and Xhol, in order to be used as vector,
- 5 and agarose gel purified.
- A 161-bp Clal/EcoRI fragment, obtained from 495-Clal-cl135M1 as well as a 254-bp EcoRI/XbaI fragment, obtained from 650-720-EcoRI-cl39, and a 374-bp XbaI/Xhol fragment, obtained from piece2-cl4, were agarose gel purified.
- Equimolar amount (0.1 pmole) of each of these 4 DNA fragments were mixed and ligated
- 10 together.
- 50µl of competent XL1Blue bacteria were transformed with 1/10th of the ligation products according to the protocole described for piece1.
- Direct colony PCR screening was performed using M13Reverse and T7 primers.

15 **Piece 4 gp160:** The strategy for building that piece is depicted in figure 15. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described above for piece1, except the following:

- The plasmid 1630-XbaI-cl2 was linearised by SacII/ EagI digestion and agarose gel
- 20 purified, in order to be used as vector.
- A 190-bp EagI/HindIII fragment, obtained from snut 1700-EagI-cl4, as well as a 177-bp SacII/HindIII fragment, obtained from snut 1890-HindIII-cl8, were agarose gel purified.
- Equimolar amount (0.1 pmoles) of the three DNA fragments described above were ligated in an one step ligation.
- 25 - 50µl of competent XL1Blue bacteria were transformed with 1/10th of the ligation products according to the protocole described for piece1.
- Direct colony PCR screening was performed using M13Reverse and T7 primers.

Piece 4-gp150: PCR-based site-directed mutagenesis was performed on double-stranded

30 plasmid-DNA from piece4-cl4 according an adaptation of the ExSite™ PCR-Based Site-Directed Mutagenesis Kit procedure (Stratagene cat#200502)(Weiner, M.P., Costa, G.L., Schoettlin, W., Cline, J., Marthur, E., and Bauer, J.C. (1994) Gene 151:119-123). The mutations introduced are shown in bold letters in the primer sequences below. PCR amplification was performed with the Expand™ High Fidelity PCR System (Boehringer

- 35 Mannheim, cat#1759078). Briefly, 1.5 µg, 0.5 µg or 0.1 µg of circular dsDNA was mixed with 1.5 pmoles of P4M2S (5'-TCTGGAAAGCTCAGGGGGCTGCATCCCTGGC-3') and 1.5

pmoles of P4M2AS (5'-CCGCCTGCCGTGTGACGGATCCAGCTCC-3') in a final volume of 50µl containing 4 dNTPs (250µM each), 1x Expand HF buffer 2 (Boehringer Mannheim, cat#1759078), 0.75µl of Enzyme mix (Boehringer Mannheim, 5U/µl). The PCR was performed with a PE9600 thermo-cycler (Perkin-Elmer Corporation) under the following 5 cycling parameters : 94°C, 2min ; 15 cycles (94 °C, 45 sec ; 68 °C, 4 min); 72°C, 7min and 4 °C, hold. PCR products were phenol:chloroform extracted and precipitated (Maniatis). Plasmid template was removed from PCR products by DpnI treatment (Biolabs)(Nelson, M., and McClelland, M., 1992) followed by ethanol-precipitation. Amplicons were resuspended in 50 µl steril water, and phosphorylated according the following procedure: 7.5 µl of amplicons 10 were mixed with 0.5µl of 100mM DTT, 1 µl of 10x pk buffer and 1 µl of pk mix enzyme (pMOSBlue blunt-ended cloning kit, Amersham , cat#RPN 5110). DNA kinasing was allowed 5min at 22°C. After heat-inactivation (10min, 75°C) of the pk enzyme, 1µl of ligase (4units, Amersham , cat#RPN 5110) was directly added to the pk reaction. The ligation was allowed overnight at 22°C. 50µl of competent XL1Blue bacteria were transformed with 1/10th of the 15 ligation reaction according to the classical protocol (Maniatis). Insertion of mutations was checked by sequencing.

Piece 4-gp140: PCR-based site-directed mutagenesis was performed on piece 4-cl4, according to the procedure described for piece4-gp150 except that the primers P4M1AS (5'- 20 TGTGTGACTGATTGAGGATCCCCACTGGC-3') and P4S (5'- AGCTTGCCCACTTGCCAGCTGGAGCAGGT-3') were used.

Snut 1265-Xhol-gp120 : PCR-based site-directed mutagenesis was performed on plasmid 1265-Xhol-cl2M1 according to the procedure described for piece4-gp150 except that the 25 primers 1265MAS (5'-CTTCTCGCGCTGCACCACCGCGCGCTTGGC-3') and 1265M2S (5'- CGCGCCTAGGGCATCGCGCTATGTTCCCTC-3') were used.

Snut 1265-Xhol-gp160/uncleaved : PCR-based site-directed mutagenesis was performed on plasmid 1265-Xhol-cl2M1 according to the procedure described for piece4-gp150 except 30 that the primers 1265MAS (5'-CTTCTCGCGCTGCACCACCGCGCGCTTGGC-3') and 1265M2S (5'-AGCGCCGTGGCATCGCGCTATGTTCCCTC-3') were used.

Snut 1465-PstI-CCG : PCR-based site-directed mutagenesis was performed on plasmid 1465-PstI-cl25 according to the procedure described for piece4-gp150 except that the 35 primers 1465MAS (5'-CTGCTTGATGCCAACACGGTCAGCTG-3') and 1465MS (5'- TGCTGCGGCCGCGCTGGCTCTAGA-3') were used.

Piece 5 : The strategy for building that piece is depicted in figure 16. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described above for piece1, except the following:

- 5 - The plasmid 2425-ES-cl2 was linearised by Clal/EcoRI digestion and agarose gel purified, in order to be used as vector.
- A 129-bp PstI/Clal fragment, obtained 2190-Clal-cl6M15, as well as a 114-bp PstI/EcorI fragment, obtained from 2330-PstI-cl8, were agarose gel purified.
- Equimolar amount (0.1 pmoles) of the three DNA fragments described above were ligated
- 10 in an one step ligation.
- 50µl of competent XL1Blue bacteria were transformed with 1/10th of the ligation products according to the protocole described for piece1.
- Direct colony PCR screening was performed using T3 (5'-ATTAACCCTCACTAAAG-3') and T7 primers.

15

piece 8 : The strategy for building that piece is depicted in figure 17. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described above for piece1, except the following:

- The plasmid piece4-cl4 was linearised by XbaI/Xhol and agarose gel purified, in order to be
- 20 used as vector.
- A 200-bp Xhol/PstI fragment, obtained from 1265-Xhol-cl2M1 as well as a 178-bp PstI/XbaI fragment, obtained from 1465-PstI-cl25 were agarose gel purified.
- Equimolar amount (0.1 pmole) of these 3 DNA fragments were mixed and ligated together.
- 50µl of competent XL1Blue bacteria were transformed with 1/10th of the ligation products
- 25 according to the protocole described for piece1.
- Direct colony PCR screening was performed using T3 and T7 primers.

piece 8-gp150 : The strategy for building that piece was identical to that of piece 8, except that piece4-cl4M3 was used as vector (figure 18).

30

piece8-gp150/ uncleaved : The strategy for building that piece is identical to that of piece 8, except that piece4 gp160-cl4M3 is used as vector and a 200-bp Xhol/PstI fragment, obtained from snut 1265-Xhol-gp160/uncleaved as well as a 178-bp PstI/XbaI fragment, obtained from snut 1465-PstI-CCG are used like inserts.

35

piece 8-gp140 : The strategy for building that piece was identical to that of piece 8, except that piece4-cl4M5 was used as vector (figure19).

piece8-gp140/ uncleaved : The strategy for building that piece is identical to that of piece 8,
 5 except that piece4-cl4M5 is used as vector and a 200-bp Xhol/PstI fragment, obtained from
 snut 1265-Xhol-gp160/uncleaved as well as a 178-bp PstI/XbaI fragment, obtained from snut
 1465-PstI-CCG are used like inserts.

Piece8-gp41 : The strategy for building that piece is depicted in figure 20. RE digestion,
 10 DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies
 were performed according the same procedures described for synBX08 gp160 gene. A 63
 bp linker is to be made according to the method described for snut 2425-ES, the minigene
 approach. Thus for 2 complementary oligonucleotides: 1265-gp41S(5'-
TCGAGgctagcGCCGTGGGCATCGCGCTATGTTCCCTCGGCTTCCCTGGGCGctgca-3') and
 15 1265-gp41AS (5'-gCCCCAGGAAGCCGAGGAAC-
ATAGCGCCGATGCCAACGGCgctagcC-3' should be annealed together. This synthetic
 linker will be directly ligated into the *Xhol* /*PstI* sites of piece8-klon13 from which the snut
 1265-Xhol-cl 2M1 would have been removed.
 20 **piece7 :** The strategy for building that piece is depicted in figure 21. RE digestion, DNA
 fragments purification, ligation as well as direct PCR screening of recombinant colonies were
 performed according the same procedures described above for piece1, except the following:
 - The plasmid piece5-cl1 was linearised by *Cla*I/*Xhol* and agarose gel purified, in order to be
 used as vector.,.
 25 - A 798-bp *Xhol*/*Sac*II fragment, obtained from piece8-cl13 as well as a 140-bp *Sac*II/*Cla*I
 fragment, obtained from 2060-*Sac*II-cl21 were agarose gel purified.
 - The ligation of the 3 fragments was performed using a vector:insert ratio of 1:1, 1:2 or 1:5.
 - 50µl of competent XL1Blue bacteria were transformed with 1/10th of the ligation products
 according to the protocol described for piece1.
 30 - Direct colony PCR screening was performed using M13Reverse and T7 primers.

Example 5: assembly of genes

synBX08 gp160 gene : The strategy for building that gene is depicted in figure 6. RE
 digestion, DNA fragments purification, ligation as well as direct PCR screening of
 recombinant colonies were performed according the same procedures described for piece1.
 35 20 µg of the expression plasmid WRG7079 were digested by *Nhe*I/*Bam*HI. Plasmid DNA-

ends were dephosphorylated by Calf Intestin Phosphatase treatment (CIP, Biolabs) (Maniatis) to avoid autoligation of any partially digested vector. CIP enzyme was heat-inactivated and removed by classical phenol-chloroforme extraction. A 1277-bp Nhel/Xhol fragment, obtained from piece3-cl27, as well as a 1194-bp Xhol/BamHI fragment, obtained 5 from piece7-cl1, were agarose gel purified. The ligation was performed using a vector:insert ratio of 1:1 or 1:2. Fifty µl of competent XL1Blue bacteria were transformed with 1/10th of the ligation product according to the protocole described for piece1. After transformation bacteria were plated on LB-kanamycin agar plates. Direct PCR colony screening was performed using the primer set WRG-F (5'-AGACATAATAGCTGACAGAC-3') and WRG-R (5'-
10 GATTGTATTCTGTCCCTCAC-3'). The nucleotide sequence was determined according the methods described above for piece1.

synBX08 gp150 gene : The strategy for building that gene is depicted in figure 5. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of
15 recombinant colonies were performed according the same procedures described for synBX08 gp160 gene. A 1277-bp Nhel/Xhol fragment, obtained from piece3-cl27, as well as a 800-bp Xhol/BamHI fragment, obtained from piece 8-gp150-cl26, were agarose gel purified and then ligated into the Nhel/BamHI WRG7079 sites. The ligation was performed using a vector:insert ratio of 1:1 or 1:2.
20 For construction of the synthetic BX08 gp150, piece4 was mutated to Piece4gp150 whereby a tyrosine -> cysteine was changed and a stop codon was introduced after the transmembrane spanning domaine (TMD), followed by a BamHI cloning site. A new piece8gp150 was constructed composed of snut1265/snut1465/piece4gp150.

25 **synBX08 gp150/uncleaved gene :** RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies are performed according the same procedures described for synBX08 gp160 gene. A 1277-bp Nhel/Xhol fragment, obtained from piece3-cl27, as well as a 800-bp Xhol/BamHI fragment, obtained from piece 8-gp150/uncleaved, are agarose gel purified and then are ligated into the Nhel/BamHI
30 WRG7079 sites. The ligation was performed using a vector:insert ratio of 1:1 or 1:2.

synBX08 gp 140 gene : The strategy for building that gene is depicted in figure 4. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described for
35 synBX08 gp160 gene. A 1277-bp Nhel/Xhol fragment, obtained from piece3-cl27, as well as a 647-bp Xhol/BamHI fragment, obtained from piece 8-gp140-cl2, were agarose gel purified

and then ligated into the Nhel/BamHI sites of WRG7079. The ligation was performed using a vector:insert ratio of 1:1 or 1:2. For construction of the synthetic BX08 gp140, piece4 was mutated to Piece4gp140 whereby a stop codon was introduced just before the TMD followed by a BamHI cloning site. A new piece8gp140 was constructed composed of

5 snut1265/1465/piece4gp140.

synBX08 gp140/uncleaved gene : RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies are performed according the same procedures described for synBX08 gp160 gene. A 1277-bp Nhel/Xhol fragment, obtained 10 from piece3-cl27, as well as a 800-bp Xhol/BamHI fragment, obtained from piece 8-gp140/uncleaved, are agarose gel purified and then ligated into the Nhel/BamHI WRG7079 sites. The ligation was performed using a vector:insert ratio of 1:1 or 1:2.

synBX08 gp 120 gene : The strategy for building that piece is depicted in figure 3. RE 15 digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described for synBX08 gp160 gene. A 1277-bp Nhel/Xhol fragment, obtained from piece3-cl27, as well as a 206-bp Xhol/BamHI fragment, obtained from 1265-Xhol-gp120-clM5, were agarose gel purified and then ligated into the Nhel/BamHI sites of WRG7079. The ligation was performed 20 using a vector:insert ratio of 1:1 or 1:2. For construction of the synthetic BX08 gp120, snut 1265 was mutated to S_{1265gp120} to introduce a stop codon at the gp120/gp41 cleavage site followed by a BamH1 cloning site.

The gp160, gp150, gp140, and gp120 genes are cloned (Nhel-BamHI) and maintained in an 25 eucaryotic expression vectors containing a CMV promotor and a tPA leader, but other expression vectors may be chosen based on other criteria e.g. antibiotic resistance selection, other leader sequences like CD5 etc, presence or not of immune stimulatory sequences etc.

SynBX08 gp41 gene : The strategy for building that gene is depicted in figure 20. RE 30 digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described for synBX08 gp160 gene. Piece 8-gp41 is ligated with snut 2060-SacII-klon21 and piece 5 as already decribed for the construction of piece 7, creating piece 7-gp41 (P_{7gp41}). Subsequently the piece 7-gp41 containing the entire gp41 gene will be cloned in WRG7079 using the Nhel 35 and BamHI sites.

Example 6a: High expression by codon optimization

To analyse the expression of glycoproteins from the wild type and synthetic BX08 envelope genes RIPA was performed on transfected mammalian cell lines. Both cell membrane associated and secreted HIV-1 glycoproteins from the cell supernatants were assayed. The 5 envelope plasmids were transfected into the human embryonic kidney cell line 293 (ATCC, Rockville, MD) or the mouse P815 (H-2D^d) cell line using calcium phosphate (CellPfect Transfection kit, Pharmacia). For radio immune precipitation assay (RIPA), transfected cells were incubated overnight, washed twice and incubated for 1 hour with DMEM lacking cysteine and methionine (Gibco). Then the medium was replaced with medium containing 50 10 µCi per ml of [³⁵S] cysteine and 50 µCi/ml of [³⁵S] methionine (Amersham Int., Amersham, UK) and incubation continued overnight. Cells were centrifuged, washed twice with HBSS and lysed in 1 ml ice-cold RIPA buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 50 mM EDTA, 1% Nonidet P-40, 0.5% sodiumdeoxycholate) to detect *membrane bound* Env glycoproteins. The cell lysates were centrifuged for 15 min. at 100,000 × g to remove any undissolved particles 15 and 100 µl immune precipitated with protein A-sepharose coupled human polyclonal IgG anti-HIV antibodies (Nielsen et al., 1987). For analysis of secreted Env glycoproteins 500 µl of the 5 ml supernatants from transfected cells were incubated with protein A-sepharose coupled human polyclonal IgG anti-HIV antibodies. After washing three times in cold RIPA buffer and once in PBS, the immuno precipitates were boiled for 4 min. in 0.05 M Tris-HCl, 20 pH 6.8, 2% SDS, 10% 2-mercaptoethanol, 10% sucrose, 0.01% bromophenol blue and subjected to SDS PAGE (Vinner et al., 1999). Electrophoresis was carried out at 80 mV for 1 hour in the stacking gel containing 10% acrylamide, and at 30 mV for 18 hours in the separating gradient gel containing 5-15% acrylamide. Gels were fixed in 30% ethanol-10% acetic acid for 1 hour, soaked for 30 min. in En3Hance (Dupont #NEF 981), washed 2 × 15 25 min. in distilled water, dried and autoradiography performed on Kodak XAR-5 film.

Transfection of human 293 cells with the syn.gp120_{BX08} and syn.gp140_{BX08} genes, respectively, resulted in high amounts of only secreted HIV-1 glycoproteins (Fig. 22a, lane 9 and 8). Thus, the synthetic gene in the absence of rev expresses the HIV-1 surface glycoprotein of the expected size which is recognised by human anti-HIV-1 antisera. The 30 expression of BX08 gp120 was Rev independent and with roughly the same high amount of gp120 from the syn.gp120MN gene (Fig. 22a, lane 2). Fig. 22a, lane 6 and lane 7 shows the expression of only membrane bound gp160 and gp150 from 293 cells transfected with syn.gp160_{BX08} and syn.gp150_{BX08} plasmids, respectively. Also transfection with wt.gp160_{BX08} plasmid resulted in a significant expression of membrane bound gp160 despite the absence 35 of Rev (Fig. 22a, lane 3). Co-transfection with equimolar amounts of Rev encoding plasmid seemed to increase this expression somewhat (Fig. 22a, lane 4). This is seen despite the

lower transfection effectivity using two plasmids and the use of only half the amount of wt.gp160_{BX08} DNA when combined with pRev. The amounts of secreted HIV-1 glycoproteins from gp120 and gp141 accumulating in the cell supernatants seemed higher than the amounts of cell associated glycoproteins at the time of harvesting of the cells. Interestingly,

5 the amounts of gp160 produced from the "humanized" gene were about equal to the amounts produced by the wt.gp160_{BX08} + pRev genes, respectively (Fig. 22a, lane 4 and 6). The processing of gp160, gp150 and gp140 into gp120 plus a gp41, or fractions of gp41, produced from wild type or synthetic genes in the 293 cell-line did not function well under these experimental conditions. Same phenomenon was seen in RIPA from 293-CD4 cells

10 and HeLa-CD4 cells infected by HIV-1_{MN} (Vinner et al., 19999). Because of the absence of CCR5 these cell-lines could, however, not be infected by HIV-1 strain BX08.

Example 6b: Radio immuno precipitation assay (RIPA) of synthetic BX08 transfected cells showing expression of glycoproteins from synthetic BX08 env plasmids

15 The synthetic envelope plasmid DNA were transfected into the human embryonic kidney cell line 293 (ATCC, Rockville, MD) using calcium phosphate (CellPfect Transfection kit, Pharmacia). For immune precipitation analysis, transfected 293 cells were treated and analysed according to the method described in example 6a.

To analyse expression from these genes, an SDS-PAGE of the ³⁵S-labelled HIV-1

20 envelopes, immune precipitated from the transfected cells is shown in figure 22b. Both cell-membrane associated and secreted HIV-1 envelope glycoproteins in the cell supernatants were assayed. Transfection of 293 cells with the synthetic BX08 gene encoding gp120 (syn.gp120BX08) in lane 4, and syn.gp140BX08 (lane 5) that did not contain rev encoding regions, resulted in abundant amounts of HIV-1 gp120. Thus, the expressions were Rev

25 independent and expressed in roughly same high amounts as the syn.gp120MN and syn.gp160MN genes (lane 3 and 2, respectively) already showed by our group and others to be markedly increased in comparison with HIV MN wild type genes including rev (Vinner et al 1999).

Transfection with syn.gp150 plasmid (lanes 7 and 8) resulted in significant expression of

30 membrane associated gp120 and low detectable amounts of truncated form of gp41 (cell pellet in lane 7) with no detectable HIV-1 glycoprotein in the cell supernatant lane 8. It is concluded that the synthetic BX08 genes express the envelope glycoproteins of expected size which are recognised by human anti-HIV-1 antiserum.

Example 6C FACS

To quantitate the surface expression of HIV glycoproteins from the wild type and synthetic BX08 envelope genes transfection experiments were done and cell surface expression

5 examined by FACS (flow cytometer).

10 µg of the BX08 envelope plasmid (wild type BX08gp160 or synBX08gp160) plus 10 µg of

an irrelevant carrier plasmid pBluescript were used to transfect a 80-90% confluent layer of

293 cells in tissue culture wells (25 cm²) using the CellPect kit (Pharmacia). After 48 hours

10 cells were Versene treated, washed and incubated with a mouse monoclonal IgG antibodies to HIV gp120 (NEA-9301, NEN™, Life-Science Products Inc., Boston) for time 30 min. on wet ice followed by washing in PBS, 3% FCS and incubation with Phyto-Erytrin (PE) labelled rat anti-mouse IgG1 (Cat #346270, Becton Dickinson) according to the manufacture. After

washing the cells were fixed in PBS, 1% paraformaldehyd, 3% FCS, and analysed on a

15 FACS (FACScan, Becton-Dicknsson). Table 5 show in duplicate expression of BX08 gp160 from 11 % of the cells transfected with wild type BX08 (number 1 and 2) compared to the 48 % of cells expression BX08 glycoprotein when transfected with the synthetic gene (number 3 and 4). Thus, a several fold higher expression is obtained using the synthetic BX08 gene.

20 **Table 5** FACS analysis of 293 cells transfected with synBX08gp160 (No 1 and 2) and wt.gp16+BX08 (No3 and 4) and stained with monoclonal antibodies to surface expressed HIV glycoproteins. A higher expression was obtained with the synthetic gene (mean 48%) as compared to the wild type gene (mean 11 %).

	50 ul	45 ul	A	B	C	C - A	C - B
1	syn.gp160BX08 +	pBluescript SK+	2,57	2,85	36,91	34,34	34,06
2	syn.gp160BX08 +	pBluescript SK+	2,83	2,14	58,42	55,59	56,28
3	wt.gp160BX08 +	pBluescript SK+	1,95	1,52	7,51	5,56	5,99
4	wt.gp160BX08 +	pBluescript SK+	2,97	1,42	14,41	11,44	12,99

A: No primary antibody added (control for unspecific secondary Ab binding)

25 B: Neither Primary Ab nor Secondary Ab added (autoflourosense control)

C: Primary Ab and secondary Ab added.

Example 6D Analyses of the surface expression and biological functionality

To analyse the surface expression and biological functionality from the wild type and

30 synthetic BX08 envelope genes transfection experiments were done and cell fusion microscopically studied using HIV envelope receptor expressing cells.

10 µg of the BX08 envelope plasmid (wt.BX08gp160 or syn.BX08gp160 or empty WRG7079 vector plasmid) plus 5 µg of a plasmid (pEGFP, Clonetech) expressing green fluorescent protein (GFP) were transfected into 2 x 10⁶ adherent U87.CD4.CCR5 cells (NIH AIDS Res. & Reference program, catalog #4035) stably expressing CD4 and CCR5, using the CellPfect 5 transfection kit (Pharmacia). After 48 hours the cells were examined by microscopy and photographed (Fig 22c).

Fig 22c panel A show the negative control (empty WRG7079 plus pGFP) giving no syncytia. Panel B show cells transfected with the wild type BX08 gp160 plasmid where cell-to-cell fusion (syncytia) is seen. Panel C show cells transfected with the same amounts of 10 synBX08gp160 plasmid and demonstrating a much higher degree of cell-cell fusion. In fact most or all of the cells in the culture plate were fused at this time. This experiment show surface expression of functional HIV gp160 with tropism to the CCR5 receptor, as well as a much higher expression and biological activity from the synthetic BX08 gene as compared to the wild type equivalent.

15

Example 7: Gene inoculation of mice for immunization

6-7 weeks old female BALB/c mice were purchased from Bomholdtgaard, Denmak. Microbiological status was conventional and the mice were maintained in groups of 4/5 per cage with food and water ad libitum and artificially lighted 12 hours per day. Acclimatization 20 period was 2 days. Mice were anaesthetized with 0.2 ml i.p. of rohypnol:stesolid (1:3, v/v) and DNA inoculated by either i.m. injection of 50 µl 2 mg/ml of plasmid DNA in each tibia anterior muscle at week 0, 9, and 15 and terminated week 18; or gene gun inoculated on shaved abdominal skin using plasmid coated gold particles (0.95 µm particles, 2 µg DNA/mg gold, 0.5 mg gold/shot, 50-71% coating efficiency) with the hand held Helios® gene gun 25 device (BioRad) employing compressed (400 psi) Helium as the particle motive force. Mice were gene gun vaccinated at week 0, 3, 6, 9, 15, and terminated week 18.

Example 8: Serological assays

Western blotting. The induction of a humoral response to gp120 and gp41 antigens by *in vivo* expression of the encoded glycoproteins from the synthetic BX08 genes was examined 30 by western immuno blotting (Figure 27). Mouse antisera (1:40) were evaluated in western blotting using the commercial HIV BLOT 2.2 strips (Genelabs Diagnostic). The conjugate was a 1:200 dilution of the alkaline phosphatase-conjugated rabbit anti-mouse IgG (Dakopatts, Glostrup, Denmark). Buffers, incubation condition and colour development were

used according to the manufacturer. In these western blotting strips the gp160 band from HIV-1 IIIB contain of an oligomeric form of gp41 in a higher concentration than the monomeric gp41 band on the strip (Genelabs Diagnostic). HIV-1_{IIIB} lysate is used in these commercial strips where the gp160 band is composed by addition of tetrameric gp41. All 5 preimmune sera tested negative in western blotting. Mice inoculated with syn.gp120_{BX08} showed antibody response to the heterologous gp120 of HIV-1 IIIB. Inclusion of the extracellular part of gp41 in the gene syn.gp140_{BX08} induced antibody reaction to both gp120 and gp41 in all mice. This confirms the *in vivo* expression of BX08 gp120 and the extracellular part of gp41. DNA vaccination with syn.gp160_{BX08} encoding the membrane 10 bound glycoprotein induced antibodies to gp120 and gp41 in 50% and 64% of the mice, respectively. DNA vaccination with syn.gp150_{BX08} induced detectable antibodies to gp120 and gp41 in 41% and 53%, respectively. Induction of different levels of antibodies could explain the difference in numbers of positive reactive mice sera in this qualitative western blotting.

15 **ELISA.** Mouse anti HIV-1 gp120 antibodies were measured by indirect ELISA. Briefly, wells of polystyrene plates Maxisorb (Nunc) were coated for 2 days at room temperature with HIV-1 IIIB recombinant gp120 (Intracel) at 0.2 µg/100 µl of carbonate buffer, pH 9.6. Before use the plates were blocked 1 hour at room temperature with 150 µl/well of washing buffer (PBS, 0.5 M NaCl, 1% Triton-X-100) plus 2% BSA and 2% skimmilk powder. After 3 x 1 min. 20 washings, mouse plasma was added at 100 µl/well diluted in blocking buffer and ELISA plates incubated for 90 min. at room temperature using a microtiter plate shaker. As standard curve we used a mouse monoclonal antibody to a conserved part of gp120 between V5-C5 (MRDNWRSELYKY) (#NEA-9301, NEN™ Life Science Products, Inc., Boston, MA). As calibration control included on each plate we used a plasma pool from 10 mice vaccinated 25 with BX08 gp120. Plates were again washed 5 x 1 min. and incubated 1 hour at room temperature with 100 µl/well of HRP-conjugated rabbit anti-mouse IgG (#P260, Dakopatts, Glostrup, Denmark) diluted 1:1000 in blocking buffer. Colour was developed with 100 µl/well of peroxidase enzyme substrate consisting of 4 mg of o-phenylenediamine in 11 ml water plus 4 µl hydrogen peroxide (30%, w/w). The enzyme reaction was terminated after 30 min. 30 by 150 µl/well of 1M H₂SO₄. The optical density (OD) of wells was measured at 492 nm using a microplate photometer (Molecular Devices, Biotech-Line, Denmark). Anti-HIV-gp120 IgG titers were expressed as the reciprocal plasma dilution resulting in an OD_{492nm} value of 0.500. Mouse anti-HIV-1 BX08 antibodies were also measured by indirect peptide ELISAs as described above using a BX08 V3 peptide (SIHIGPGRAFYTTGD) (Schafer, Copenhagen, 35 Denmark).

The IgG antibody response to HIV-1_{III B} rgp120 quantitated by ELISA is seen in Fig. 28 and Fig. 29. No background activity was observed in preimmune sera or in sera from 4 mice immunized with empty WRG7079 vector in parallel with the BX08 genes. All mice inoculated with the synthetic BX08 genes either by gene gun or by i.m. injection responded and showed 5 a persistent and high titered (about 100-10,000) IgG response to rgp120 as exemplified in Fig. 4. When comparing the median titers for groups of mice (Fig. 29) a moderate antibody response was observed with the wt.gp160_{BX08}. Intramuscular and gene gun immunization with a mixture of wt.gp160_{BX08} plasmid plus Rev encoding plasmid did not increase this antibody response. This was found even when both plasmids were coated onto the same 10 gold particles to ensure co-transfection of single target cells. However, to ensure inoculation of equal amounts of total DNA only half of the amount of wt.BX08 plasmid was used when mixing with pRev which may have contributed to the lower antibody response when pRev was included. A 5-fold improvement of the antibody response was obtained using the 15 syn.gp160_{BX08} gene. This antibody response seemed further improved using the syn.gp150_{BX08} gene where the cytoplasmic internalization signals were eliminated but only using gene gun inoculation. For both the gene gun inoculation of skin and i.m injection the highest antibody titers to rgp120 were induced by genes encoding *secreted* gp120/gp140 glycoproteins versus *membrane bound* gp150/gp160 glycoproteins, respectively. In general, equal antibody and ELISA titers to rgp120 were obtained using gene gun and i.m. injection of 20 the BX08 vaccine genes.

Example 9: Neutralization assay

Mouse plasma was diluted in culture medium (RPMI-1640 medium (Gibco) supplemented with antibiotics (Gibco), Nystatin (Gibco) and 10% FCS (Bodinco)) and heat inactivated at 60°C for 30 min. Of the HIV-1 strain BX08 (50 TCID₅₀ per ml propagated in PBMC) 250 µl 25 was incubated for 1 hour at room temperature with 250 µl dilution of mouse serum (four five-fold dilutions of mouse serum, final dilutions 1:20 to 1:2500). After incubation 1 × 10⁶ PBMC in 500 µl culture medium was added to the virus-serum mixture and incubated overnight at 37°C in 5% CO₂. Subsequently, eight replicates of 10⁵ PBMC in 200 µl culture medium were cultured in 96-well culture plates (Nunc) at 37°C in 5% CO₂. After seven days in culture the 30 concentration of HIV antigen in the culture supernatant was quantitated using HIV antigen detection ELISA (Nielsen et al., 1987).

This ELISA is performed using human IgG, purified from high titered patient sera, both as capture antibody and biotin-linked as detecting antibody. In brief, anti-HIV-capture IgG diluted 1:4000 in PBS, 100 µl/well, are coated onto Immunoplates (Nunc) overnight at 4°C. After 35 washing five times in washing buffer 100 µl of supernatants are applied and incubated overnight

at 4°C. Plates are washed 5 times before incubation with 100 µl HIV-IgG conjugated with biotin diluted 1:1000 in dilution buffer, plus 10% HIV-1 sero-negative human plasma for 3 hours at room temperature. Five times 1 min. washing in washing buffer are followed by 30 min. incubation with 100 µl of 1:1000 avidine-peroxidase (Dako P347 diluted in dilution buffer). Six 5 times 1 min. washings, 5 in washing buffer and the last one are done in dH₂O before colour is developed with 100 µl of peroxidase enzyme substrate consisting of 4 mg of OPD in 11 ml water plus 4 µl hydrogen peroxide (30 %, w/w). The enzyme reaction is terminated after 30 minutes by additional 150 µl of 1M H₂SO₄.

The HIV antigen concentration in cultures, preincubated with mouse serum, was expressed 10 relatively to cultures without mouse serum (culture medium), and the percentage inhibitions of the different dilutions of mouse serum were calculated. The 50% inhibitory concentration (IC₅₀) for each mouse serum was determined by interpolation from the plots of percent inhibition versus the dilution of serum, and the neutralizing titer of the serum was expressed as the reciprocal value of the IC₅₀. In each set-up a human serum pool known to neutralise 15 other HIV-1 strains was included in the same dilutions as the mouse serum as a calibratin control. For assay of neutralization of the heterologous SHIV89.6P the MT-2-cell-killing format was used (Crawford et al., 1999). The assay stock of SHIV89.6P was grown in human PBMC.

The neutralizing IC₅₀ antibody titers of plasma pools from 10 mice from each group 20 were measured at different time points (week 0, 9, and 18). A positive background in some preimmune sera and thus in all week 0 serum pools was noted even after dilution and heat inactivation that was found earlier to lower this background. In general the neutralizing titers to BX08 virus of such serum pools were transient and low ranging from 1:6-1:150 above background (data not shown). A possible cross-neutralization reaction to a heterologous, 25 primary HIV-1 envelope was tested using the SHIV89.6P which is relevant in macaque models of AIDS and serum pools from mice DNA immunized i.m. with syn.gp140_{BX08}. Preimmune serum had a titer of 1:37, which is indicative of a slightly positive background, whereas the 18 week p.i. serum had a positive neutralizing titer of 1:254 above background.

30 Example 10: CTL assay

The cellular immune response in mice following gene gun or i.m. genetic immunization with the different vaccine plasmids were examined (Fig. 26). Spleen was removed aseptically and gently homogenised to single cell suspension, washed 3 times in RPMI-1640 supplemented with 10% FCS and resuspended to a final concentration of 5 x 10⁷ cell/ml. The cells were 35 then incubated 5 days with mitomycin-C treated (50 µg/ml for 1 hour) mouse P815 (H-2D^d)

stimulator cells at a ratio of 10:1 in medium supplemented with 5×10^{-5} M β -mercaptoethanol. For assay of CTL response to HIV-1 BX08, P815 stimulator cells and target cells were pulsed with 20 μ g/ml of the HIV-1 BX08 V3 peptide containing a conserved murine H-2D^d restricted CTL epitope (IGPGRAFYTT) (Lapham et al., 1996). After 5 stimulation, splenocytes were washed three times with RPMI-1640 supplemented with 10% FCS and resuspended to a final concentration of 5×10^6 cells/ml. 100 μ l of cell suspension was added in triplicate to U-bottom 96-well microtiter plates and a standard 4 hour ^{51}Cr -release assay performed (Marker et al., 1973).

All synthetic BX08 plasmids induced a high specific CTL response thus confirming the *in vivo* 10 expression and *in vivo* immunogenicity. The highest CTL response was obtained with syn.gp150_{BX08} followed by syn.gp120_{BX08}-syn.gp140_{BX08}, and syn.gp160_{BX08}, respectively. Thus, the CTL response induced did not correlate with the antigen being secreted or not. However, i.m. DNA immunization with syn.gp150_{BX08} containing six putative CpG motifs 15 induced a higher CTL response than gene gun immunization (Fig. 26). This difference could be explained by the high amount of DNA used in the i.m. injections.

The T-lymphocyte cytokine profile of spleen cells after ConA stimulation as well as serum antibody IgG_{2a}/IgG₁ at week 18 were investigated. Neither the IFN γ /IL-4 nor the IgG_{2a}/IgG₁ ratios, which both reflects a Th1-type of immune response, were significantly higher for the i.m. immunized mice when compared with gene gun immunized mice (student t-test and 20 Mann-Withney U-test). Thus, the CTL response did not correlate with a certain Th-type of response and the DNA immunization technique did not bias the immune response using synthetic BX08 genes.

Example 11: Antibody responses to DNA vaccination with synBX08 env plasmid

25 A relatively low and variable antibody response (1 of 10 mice) was obtained with gene gun inoculation of the syn.gp140BX08 plasmid vaccine starting at week 9, figure 23, right panel. A higher numbers of responders 3/10 with high IgG1 antibody responses at an earlier onset (week 3-9) was obtained with the syn.gp140BX08 plasmid using i.m. injection, left panel. Sera from later time points may show more responders and/or higher titers but are not 30 assayed. However, these results show the induction of an antibody response to the BX08 V3 peptide by DNA vaccination using one of the described synthetic BX08 constructs.

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Claims

1. A method for producing a nucleotide sequence construct comprising the following steps:
 - a) obtaining a first nucleotide sequence of an HIV gene from a patient within the first 12 months of infection;
 - b) designing a second nucleotide sequence utilising the most frequent codons from mammalian highly expressed proteins to encode the same amino acid sequence as the first nucleotide sequence of a) encodes
 - c) redesigning the second nucleotide sequence of b) so that restriction enzyme sites surrounds the regions of the nucleotide sequence which encode functional regions of the amino acid sequence and so that selected restriction enzyme sites are removed thereby obtaining a third nucleotide sequence encoding the same amino acid sequence as the first and the second nucleotide sequence of a) and b) encode;
 - d) redesigning the third nucleotide sequence of c) so that the terminal snuts contain convenient restriction enzyme sites for cloning into an expression vehicle;
 - e) producing the snuts between restriction enzyme sites of c) and terminal snuts of d);
 - f) assembling the snut of step e) to form a nucleotide sequence construct.
2. A method according to claim 1, wherein the HIV gene is the gene encoding the envelope.
3. A method according to claim 1 or 2, wherein the HIV gene encodes one or more Gag proteins.
4. A method according to any of the preceding claims, wherein the HIV in step a) is in group M, O or N
5. A method according to claim 4, wherein the HIV is a group M virus.
6. A method according to any of the preceding claims, wherein the HIV is subtype A, B, C, D, E, F, G, H, I, or J.
7. A method according to claim 6, wherein the HIV is subtype B.
8. A method according to any of the preceding claims wherein the first nucleotide sequence is obtained by direct cloning.

9. A method according to any of the preceding claims, wherein the HIV in step a) is isolated with the first 11 months of infection, such as 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0.5 month after infection.

5

10. A method according to any of the preceding claims, wherein the redesigning in step c) is carried out after the second nucleotide sequence of step b) has been divided into pieces, so that each piece comprises only different restriction enzyme sites.

10 11. A method according to claim 10, wherein the second nucleotide sequence of step b) is divided into 9 pieces, or 8, or 7, or 6, or 5, or 4, or 3, or 2 pieces.

12. A method according to claim 11, wherein the second nucleotide sequence of step b) is divided into 3 pieces.

15

13. A method according to any of the preceding claims, wherein the second nucleotide sequence of step b) is designed utilising the most frequent codons from human highly expressed proteins to encode the same amino acid sequence as the first nucleotide sequence of step a) encodes.

20

14. A nucleotide sequence construct obtainable by the method of any of claims 1-13.

15. A nucleotide sequence construct according to claim 14, wherein the nucleotide sequence encoding the amino acid sequence in the first variable region is surrounded by *EcoRV* and *PstI* restriction enzyme sites.

25

16. A nucleotide sequence construct according to claims 14 or 15, wherein the nucleotide sequence encoding the amino acid sequence in the second variable region is surrounded by *PstI* and *ClaI* restriction enzyme sites.

30

17. A nucleotide sequence construct according to any of claims 14-16, wherein the nucleotide sequence encoding the amino acid sequence in the third variable region is surrounded by *ClaI* and *EcoRI* restriction enzyme sites.

18. A nucleotide sequence construct according to any of claims 14-17, wherein the nucleotide sequence encoding the amino acid sequence in the transmembrane spanning region is surrounded by *HindIII* and *SacII* restriction enzyme sites.

5 19. A nucleotide sequence construct according to any of claims 14-18, wherein the nucleotide sequence encoding the amino acid sequence on both sites of the cleavage site is surrounded by *PstI* and *XbaI* restriction enzyme sites.

10 20. A nucleotide sequence construct in isolated form which has a nucleotide sequence with the general formula (I), (II), (III), or (IV) or subsequences thereof

(I) $P_1-S_{495\text{Clal}}-S_{650-720\text{EcoRI}}-P_2-S_{1265\text{gp120}}$

(II) $P_1-S_{495\text{Clal}}-S_{650-720\text{EcoRI}}-P_2-S_{1265\text{Xhol}}-S_{1465\text{PstI}}-P_{4\text{gp140}}$

(III) $P_1-S_{495\text{Clal}}-S_{650-720\text{EcoRI}}-P_2-S_{1265\text{Xhol}}-S_{1465\text{PstI}}-P_{4\text{gp150}}$

(IV) $P_1-S_{495\text{Clal}}-S_{650-720\text{EcoRI}}-P_2-S_{1265\text{Xhol}}-S_{1465\text{PstI}}-P_{4\text{gp160}}-S_{2060\text{SacII}}-P_5$

15 wherein P_1 designates the nucleotide sequence SEQ ID NO:41, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 90% thereto;

wherein $S_{495\text{Clal}}$ designates the nucleotide sequence SEQ ID NO: 7, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 95% thereto;

20 wherein $S_{650-720\text{EcoRI}}$ designates the nucleotide sequence SEQ ID NO: 9, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 95% thereto;

wherein P_2 designates the nucleotide sequence SEQ ID NO: 43, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;

25 wherein $S_{1265\text{gp120}}$ designates the nucleotide sequence SEQ ID NO: 19, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 70% thereto;

30 wherein $S_{1265\text{Xhol}}$ designates the nucleotide sequence SEQ ID NO: 17, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 80% thereto;

35 wherein $S_{1465\text{PstI}}$ designates the nucleotide sequence SEQ ID NO: 23, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 90% thereto;

wherein P_{4gp140} designates the nucleotide sequence SEQ ID NO: 57, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;

5 wherein P_{4gp150} designates the nucleotide sequence SEQ ID NO: 55, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;

wherein P_{4gp160} designates the nucleotide sequence SEQ ID NO: 53, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;

10 wherein $S_{2060SacII}$ designates the nucleotide sequence SEQ ID NO: 33, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 98% thereto; and

wherein P_5 designates the nucleotide sequence SEQ ID NO: 59, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85%
15 thereto.

21. A nucleotide sequence construct according to claim 20, with the formula (I)

(I) $P_1-S_{495ClaI}-S_{650-720EcoRI}-P_2-S_{1265gp120}$

20 22. A nucleotide sequence construct according to claim 20, with the formula (II)

(II) $P_1-S_{495ClaI}-S_{650-720EcoRI}-P_2-S_{1265Xhol}-S_{1465PstI}-P_{4gp140}$

23. A nucleotide sequence construct according to claim 20, with the formula (III)

(III) $P_1-S_{495ClaI}-S_{650-720EcoRI}-P_2-S_{1265Xhol}-S_{1465PstI}-P_{4gp150}$

25

24. A nucleotide sequence construct according to claim 20, with the formula (IV)

(IV) $P_1-S_{495ClaI}-S_{650-720EcoRI}-P_2-S_{1265Xhol}-S_{1465PstI}-P_{4gp160}-S_{2060SacII}-P_5$

25. A nucleotide sequence construct according to claim 20 consisting essentially of the

30 subsequence P_1 .

26. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence $S_{495ClaI}$.

35 27. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence $S_{650-720EcoRI}$.

28. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence P₂.
- 5 29. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence S_{1265gp120}.
30. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence S_{1265Xhol}.
- 10 31. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence S_{1465PstI}.
32. A nucleotide sequence construct according to claim 20 consisting essentially of the
15 subsequence P_{4gp140}.
33. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence P_{4gp150}.
- 20 34. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence P_{4gp160}.
35. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence S_{2060SacII}
- 25 36. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence P₅.
37. A nucleotide sequence construct with a sequence identity of more than 85% to the
30 nucleotide sequence construct in any of claims 20-35.
38. A nucleotide sequence construct according to claim 37, wherein the sequence identity is more than 90% such as more than 95%, 98%, or 99%.
- 35 39. A nucleotide sequence construct according to claim 37, wherein the sequence identity is 100%.

40. A nucleotide sequence construct according to any of claims 14-39, coding for an HIV envelope or parts thereof with an improved immunogenicity obtained by mutating the nucleotide sequence construct of any of claims 14-39 such that one or more glycosylation sites in the amino acid sequence have been removed.

5

41. A nucleotide sequence construct according to claim 40 with a mutation at positions A307C + C309A and/or A325C + C327G and/or A340C + C342A and/or A385C + C387A and/or A469C + C471A or any combination of those.

10

42. A nucleotide sequence construct according to any of claims 14-41, coding for an HIV envelope or parts thereof with a binding site for the CXCR4 co-receptor in the third variable region.

15

43. A nucleotide sequence construct according to claim 42 with a mutation at positions G865C + A866G.

44. A nucleotide sequence construct according to any of claims 14-43, coding for an HIV envelope or parts thereof, wherein an immunodominant epitope has been modified.

20

45. A nucleotide sequence construct according to claim 44, wherein an immunodominant epitope in the third variable region has been modified.

46. A nucleotide sequence construct according to claim 45 with a deletion of nucleotides 25 793-897.

47. A nucleotide sequence construct according to claim 44, wherein an immunodominant epitope has been removed from gp41.

30

48. A nucleotide sequence construct according to any of claims 14-47, coding for an HIV envelope or parts thereof, wherein the cleavage site between gp41 and gp120 is removed.

35

49. A nucleotide sequence construct according to claim 48 with a mutation at position C1423A.

50. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence P₁, S_{495ClaI}, S_{650-720EcoRI}, and P₂.
51. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence S_{1265Xhol}, S_{1465PstI}, and P_{4gp140}.
52. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence S_{1265Xhol}, S_{1465PstI}, P_{4gp160}, S_{2060SacII}, and P₅.
- 10 53. A nucleotide sequence construct according to any of claims 14-52, further comprising a nucleotide sequence repeat coding for a functional region of the amino acid sequence.
54. A nucleotide sequence construct according to claim 53, wherein the nucleotide sequence repeat codes for amino acids in the third variable region.
- 15 55. A nucleotide sequence construct according to any of claim 14-54, further comprising a nucleotide sequence coding for a T-helper cell epitope containing sequence.
56. An expression vehicle selected from a group of viral vectors consisting of simliki forest virus (sfv), adenovirus and Modified Vaccinia Virus Ankara (MVA), further comprising a nucleotide sequence construct according to any of claim 14-55.
- 20 57. A method of individualised immunotherapy wherein the virus from a newly diagnosed patient is directly cloned, the envelope is produced with highly expressed codons, inserted into any of the nucleotide sequence constructs of claims 14-55, and administered to the patient.
58. Use of a nucleotide sequence construct according to any of claims 14-55 in medicine.
- 30 59. Use of a nucleotide sequence according to any of claims 14-55 for the manufacture of a vaccine for the prophylactics of infection with HIV in humans.
60. Use of a nucleotide sequence according to any of claims 14-55 for the manufacture of a composition for the treatment of an HIV infection in a human within 24 weeks of primary infection.

61. Use of the nucleotide sequence according to any of claims 14-55 for the production of a recombinant protein.

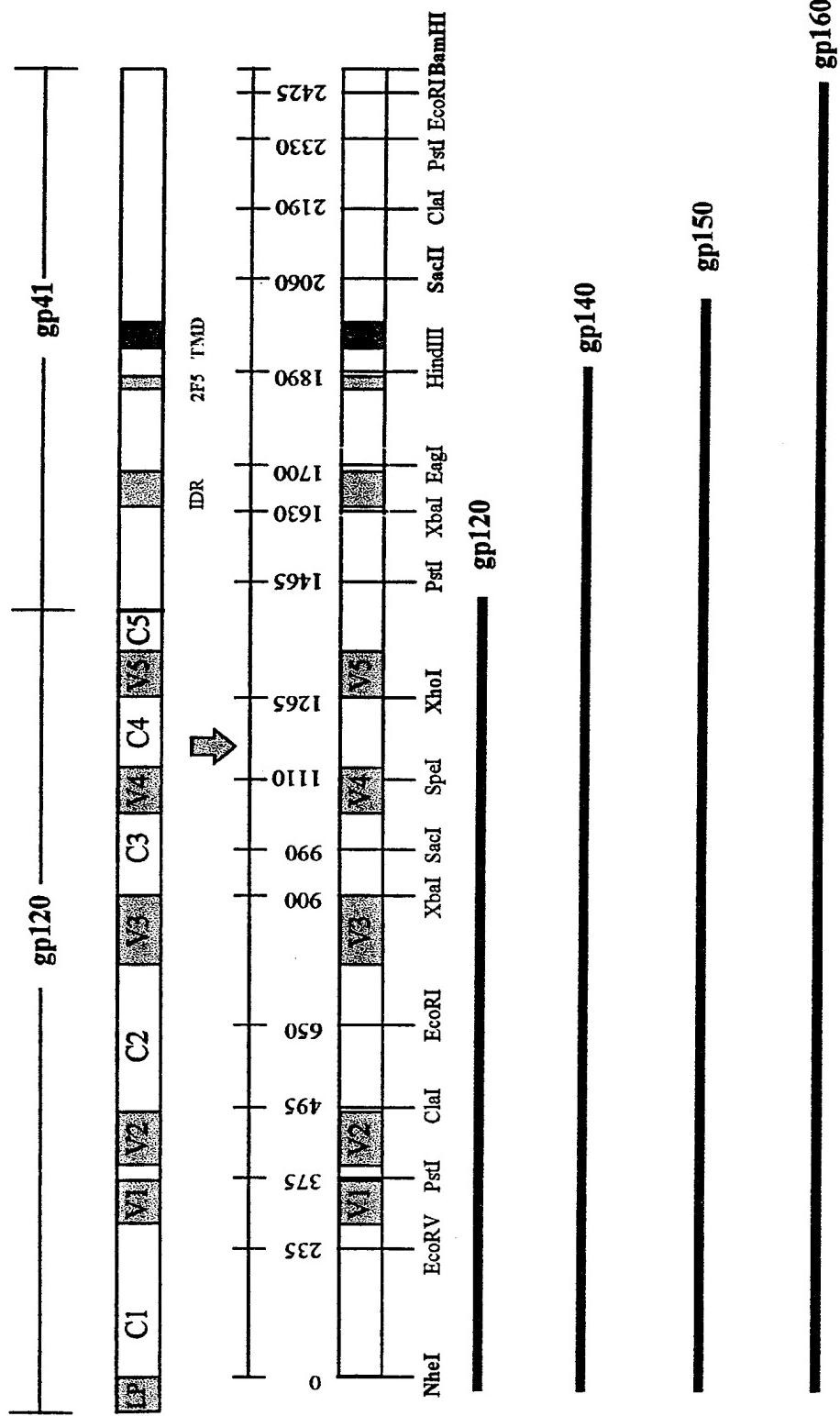
1/33

Amino acid	One letter amino acid code	Three letter amino acid code	Codon
Alanine	A	Ala	GCC
Arginine	R	Arg	CGC
Asparagine	N	Asn	AAC
Aspartic acid	D	Asp	GAC
Cysteine	C	Cys	TGC
Glutamine	Q	Gln	CAG
Glutamic acid	E	Glu	GAG
Glycine	G	Gly	GGC
Histidine	H	His	CAC
Isoleucine	I	Ile	ATC
Leucine	L	Leu	CTG
Lysine	K	Lys	AAG
Proline	P	Pro	CCC
Phenylalanine	F	Phe	TTC
Serine	S	Ser	AGC
Threonine	T	Thr	ACC
Tyrosine	Y	Tyr	TAC
Valine	V	Val	GTG

Fig. 1

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Synthetic BX08 Env
Strategy for building the full-length gp160 and derived truncated forms

**Fig. 2**

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Building of Synthetic BX08 Env (gp120)

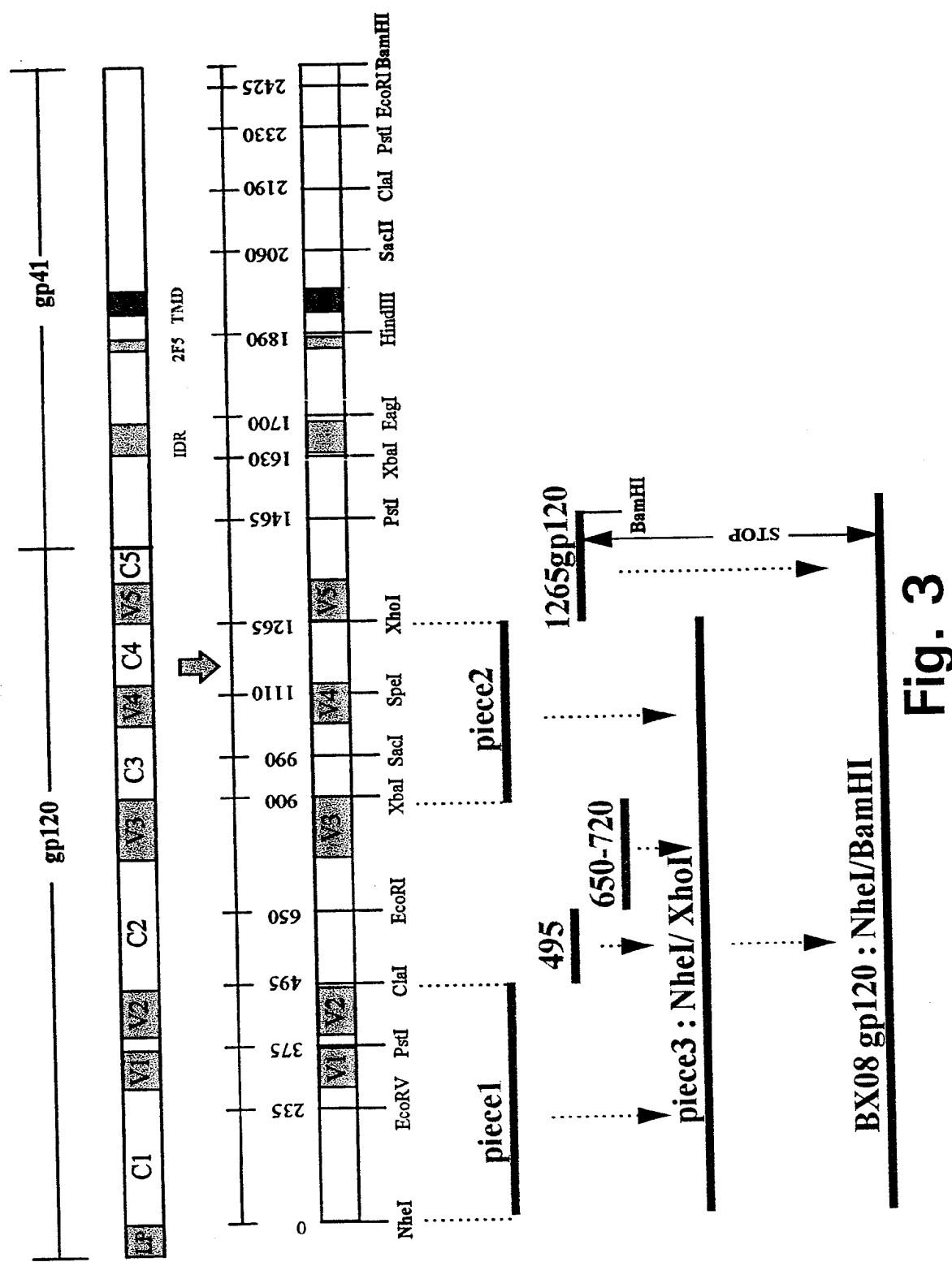
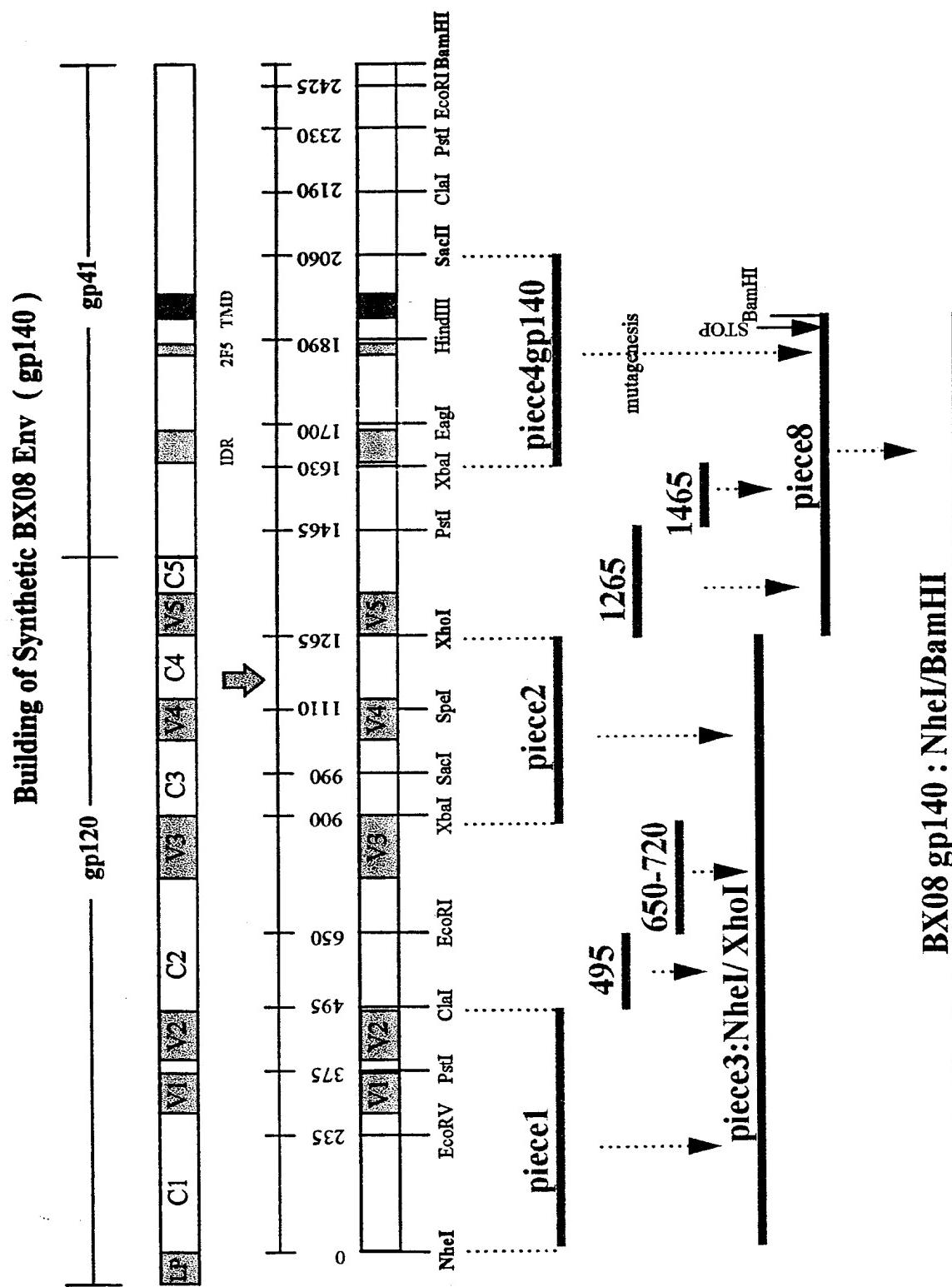
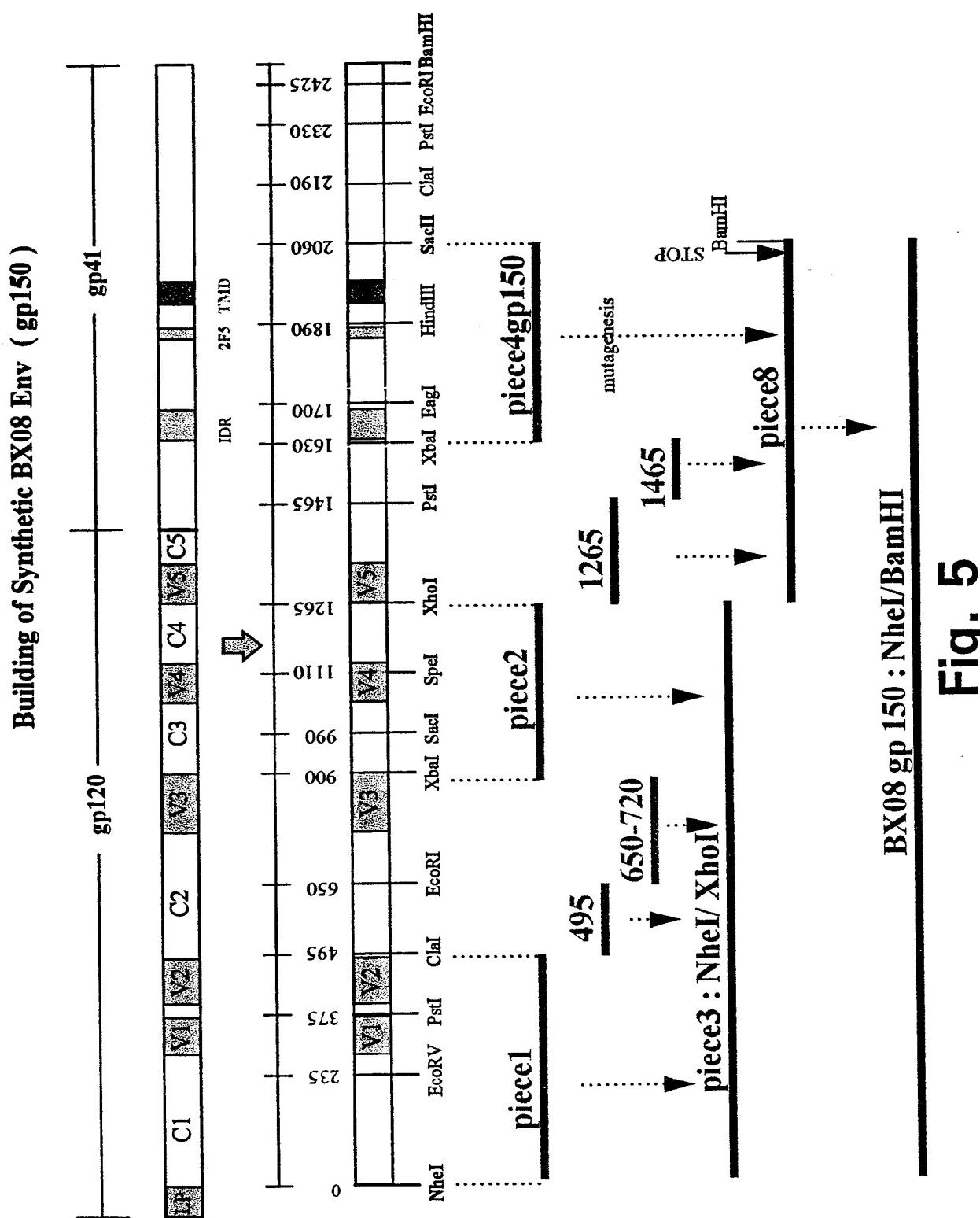


Fig. 3

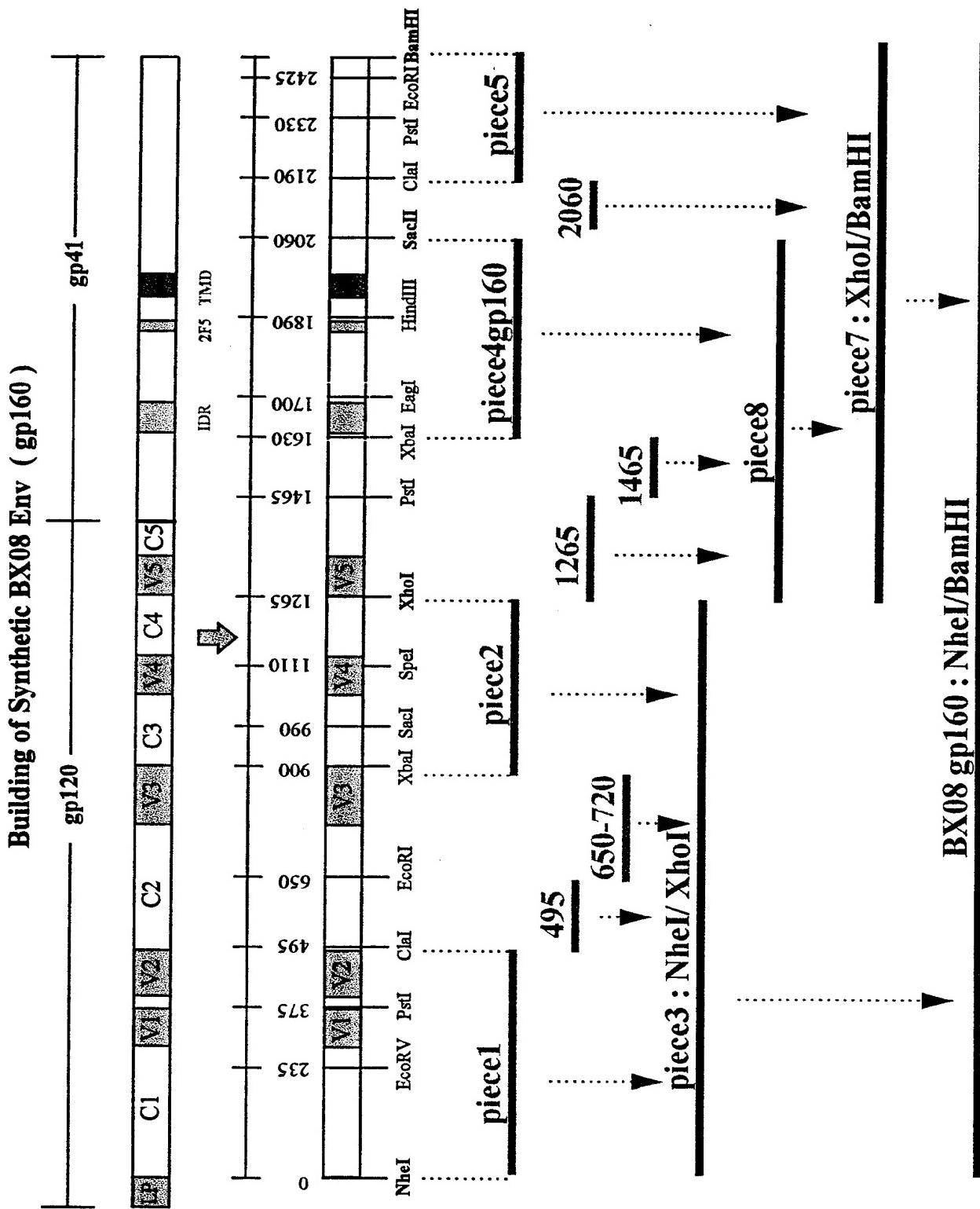
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**Fig. 4****Fig. 4**

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**Fig. 5**

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**Fig. 6**

STARTS: ATG **7/33**

aa	Σ	codons
A Ala	GCX	GCT GCC GCG GCA
C Cys	TGY	TGT TGC
D Asp	GAY	GAT GAC
E Glu	GAR	GAG GAA
F Phe	TTY	TTT TTC
G Gly	GGX	GGT GGC GGG GGA
H His	CAY	CAT CAC
I Ile	ATH	ATT ATC ATA
K Lys	AAR	AAG AAA
L Leu	YTX	TTG TTA CTT CTC CTG CTA
M Met	ATG	ATG
N Asn	AAY	AAT AAC
P Pro	CCX	CCT CCC CCG CCA
Q Gln	CAR	CAG CAA
R Arg	MGX	CGT CGC CGG CGA AGG AGA
S Ser	WSX	TCT TCC TCG TCA AGT AGC
T Thr	ACX	ACT ACC ACG ACA
V Val	GTX	GTT GTC GTG GTA
W Trp	TGG	TGG
Y Tyr	TAY	TAT TAC
	• TRR	TGA TAG TAA
X ??		

		2nd					
		T	C	A	G	3'	
T	Phe	Ser	Tyr	Cys	T		
	Phe	Ser	Tyr	Cys	C		
	Leu	Ser	•	•	A		
	Leu	Ser	•	Trp	G		
C	Leu	Pro	His	Arg	T		
	Leu	Pro	His	Arg	C		
	Leu	Pro	Gln	Arg	A		
	Leu	Pro	Gln	Arg	G		
1st	Ile	Thr	Asn	Ser	T		
	Ile	Thr	Asn	Ser	C		
	Ile	Thr	Lys	Arg	A		
	> Met	Thr	Lys	Arg	G		
A	Val	Ala	Asp	Gly	T		
	Val	Ala	Asp	Gly	C		
	Val	Ala	Glu	Gly	A		
	Val	Ala	Glu	Gly	G		

3rd

Fig. 7

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Overlapping PCR Strategy: Snut O-N-LANG (249 bases)

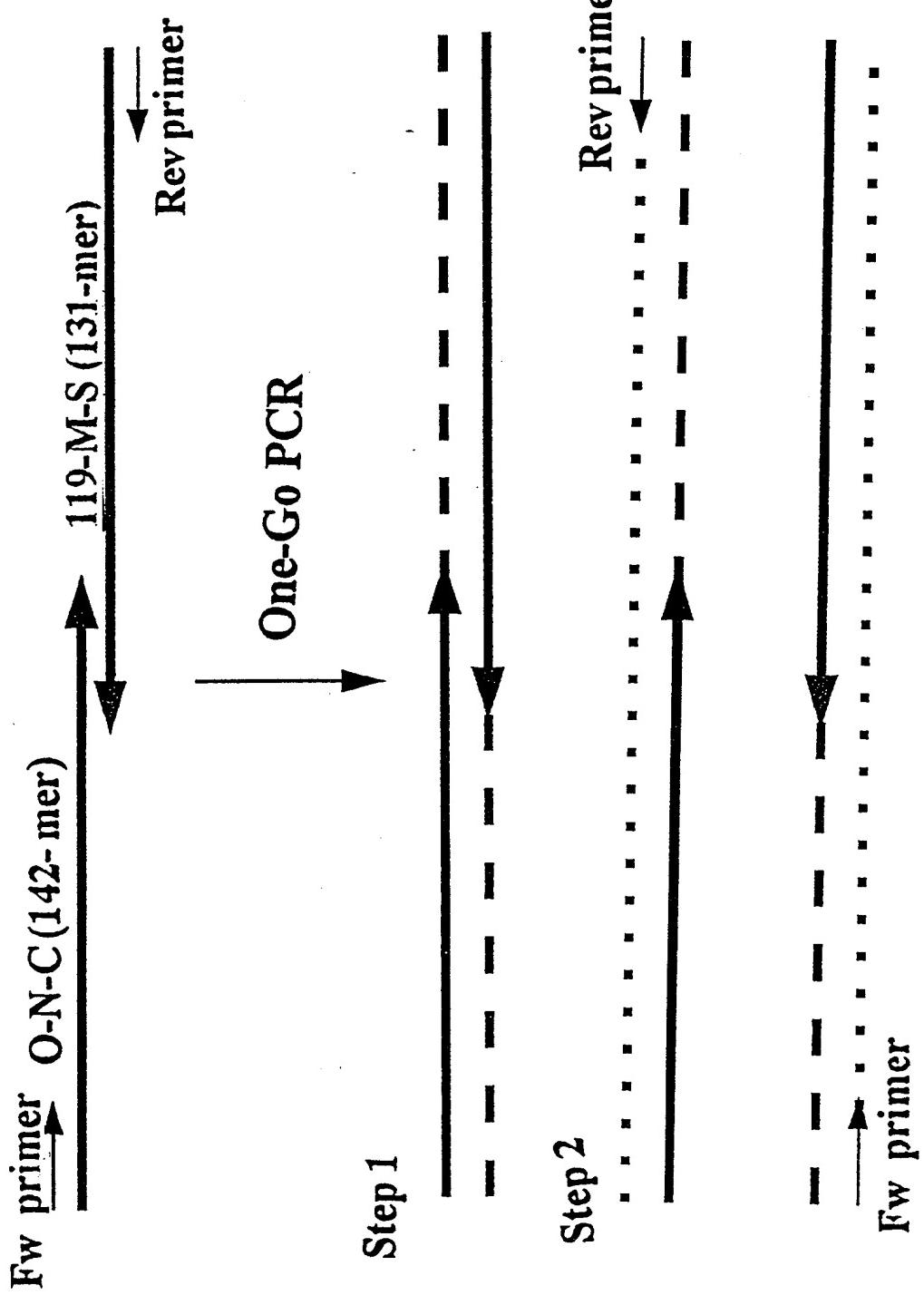


Fig. 8

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PCR approach using conserved flanking sequences

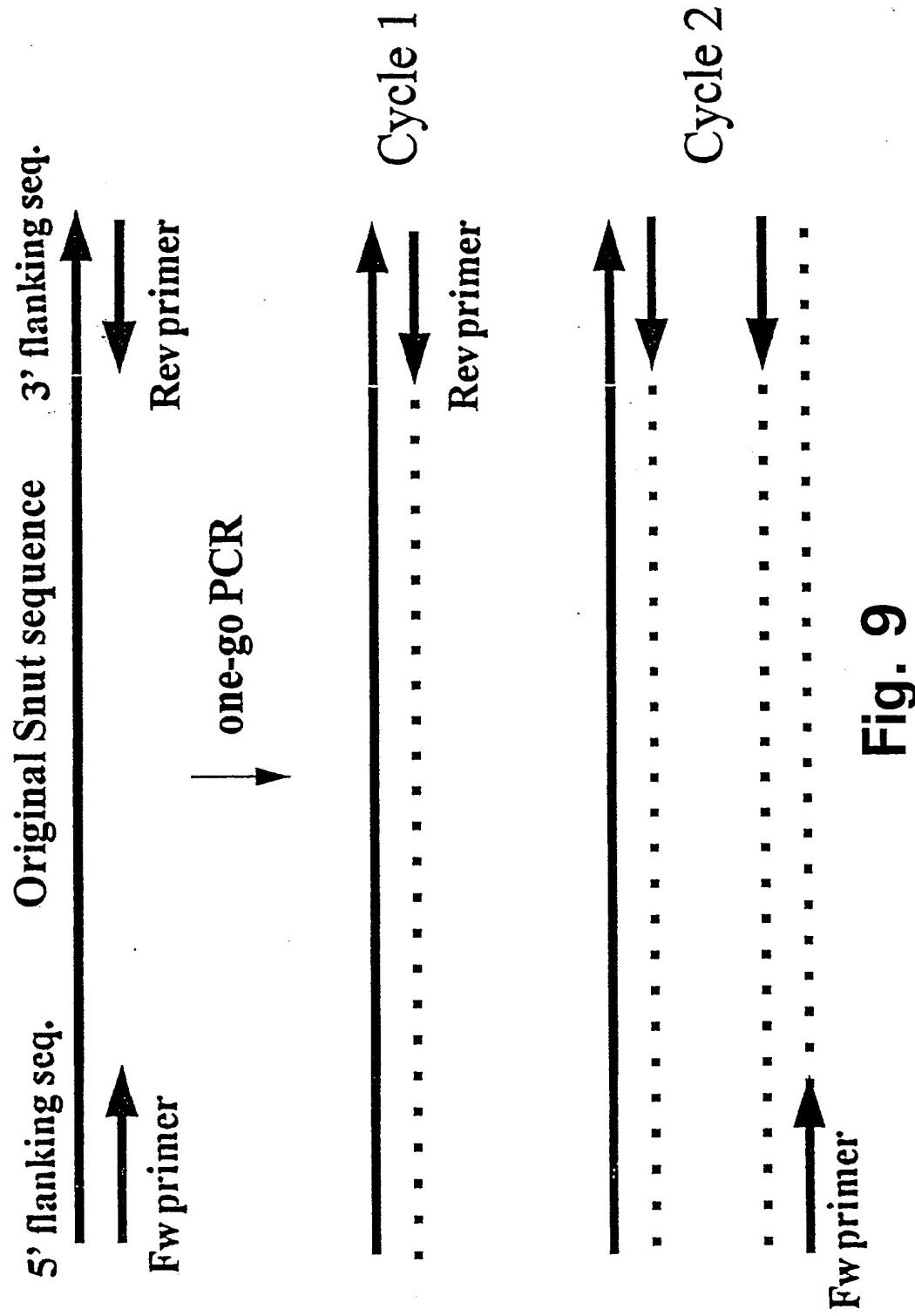


Fig. 9

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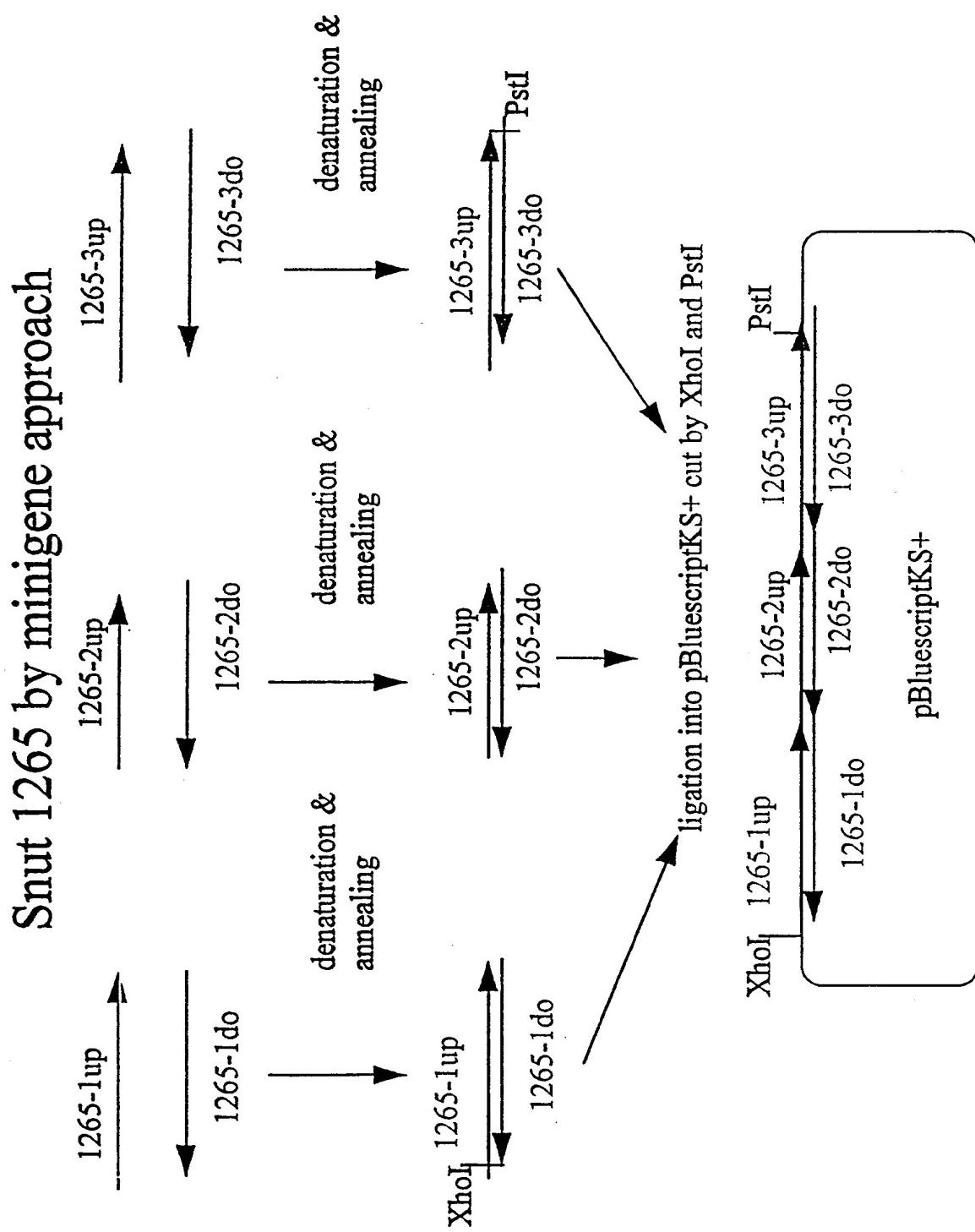
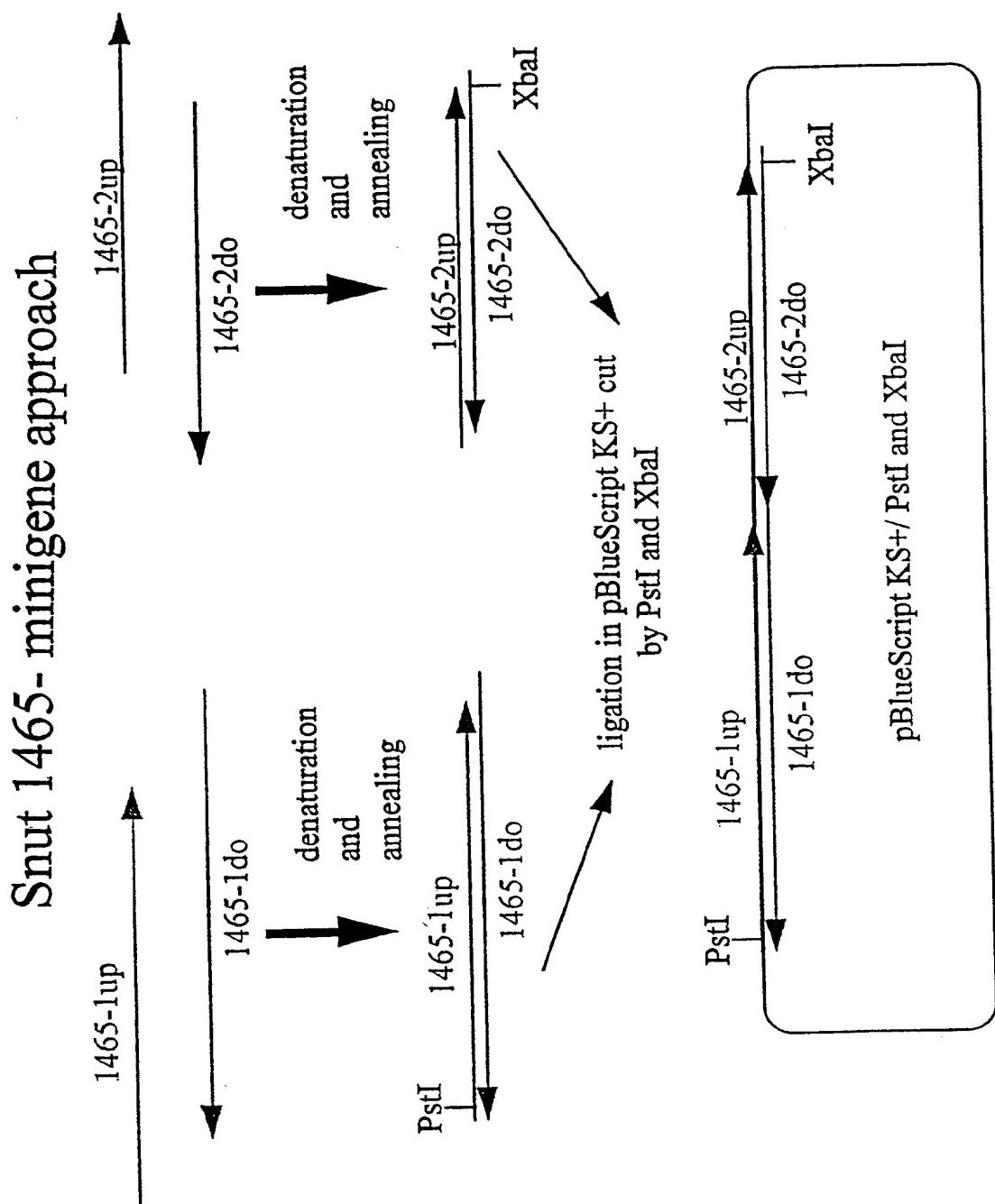


Fig. 10

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**Fig. 11**

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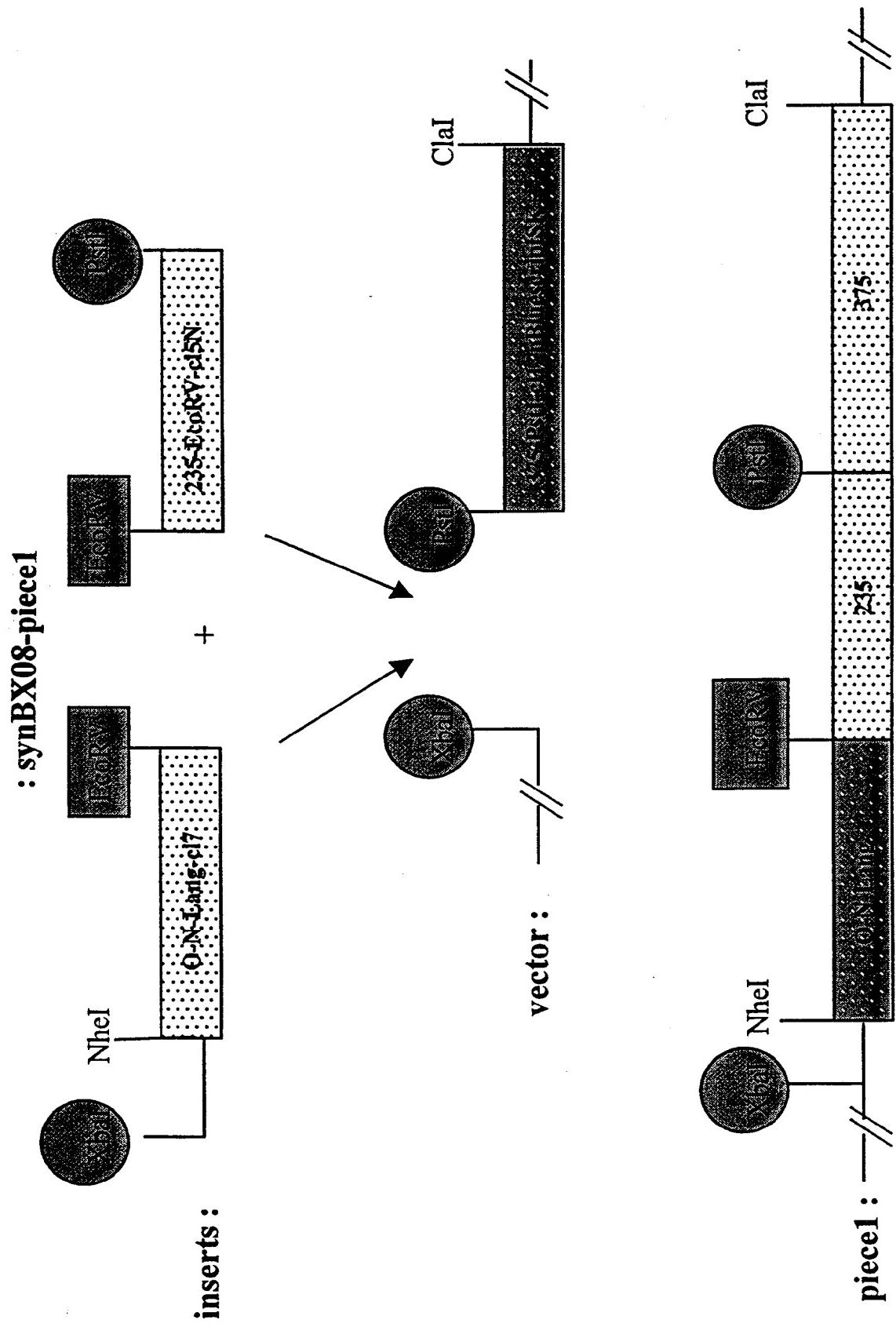


Fig. 12

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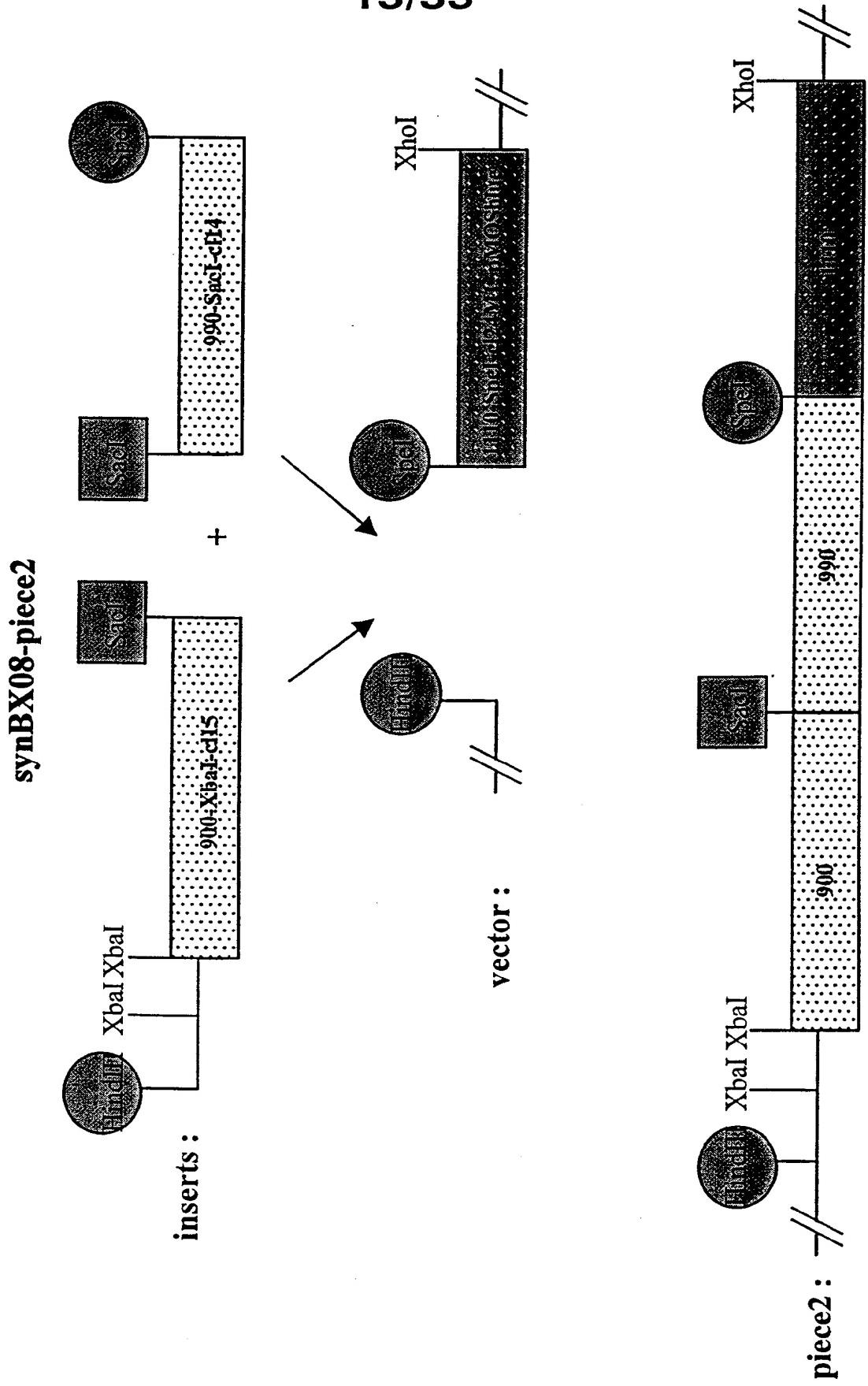


Fig. 13

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symBX08-piece3

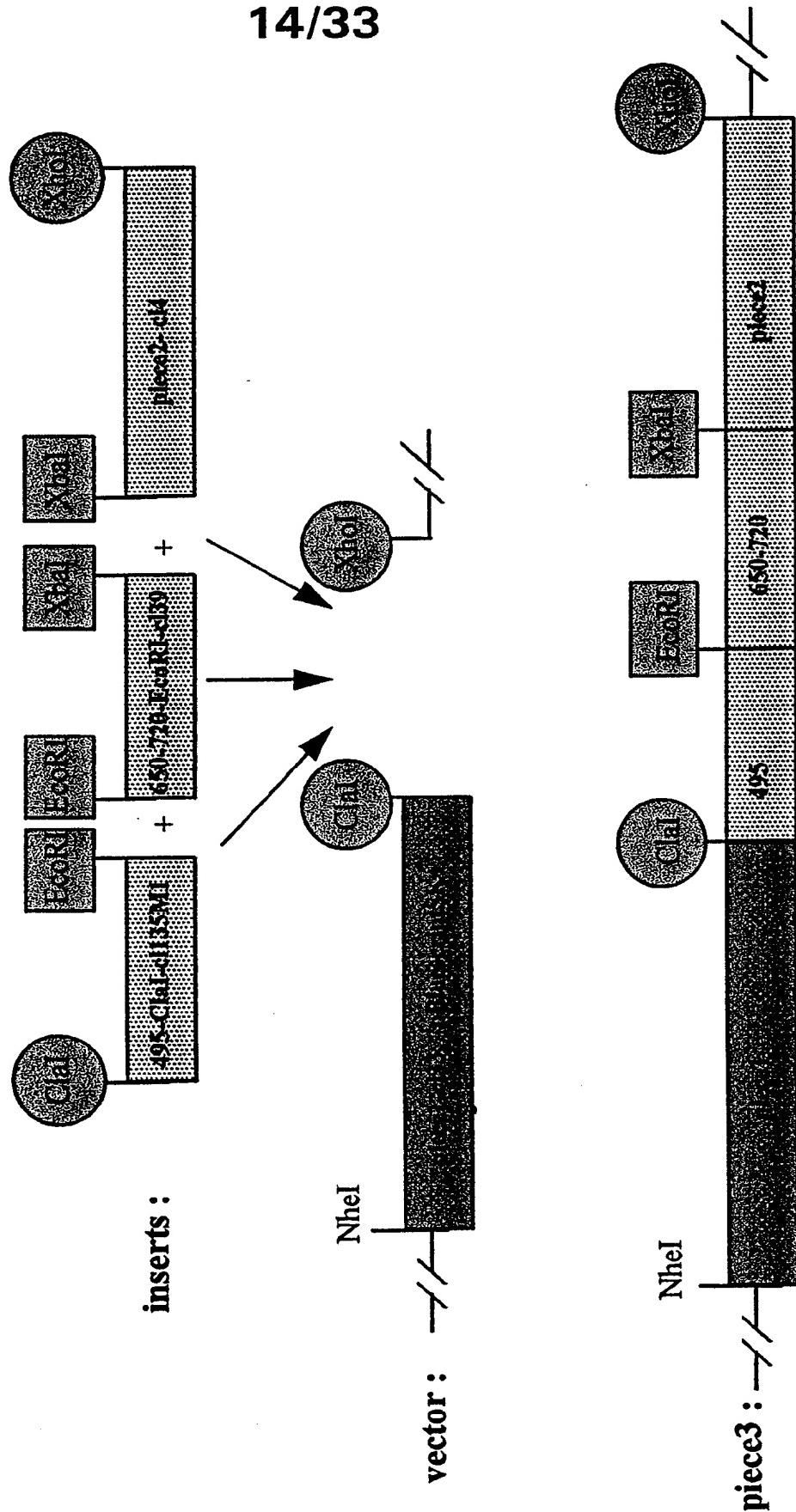


Fig. 14

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synBX08-piece4gp160

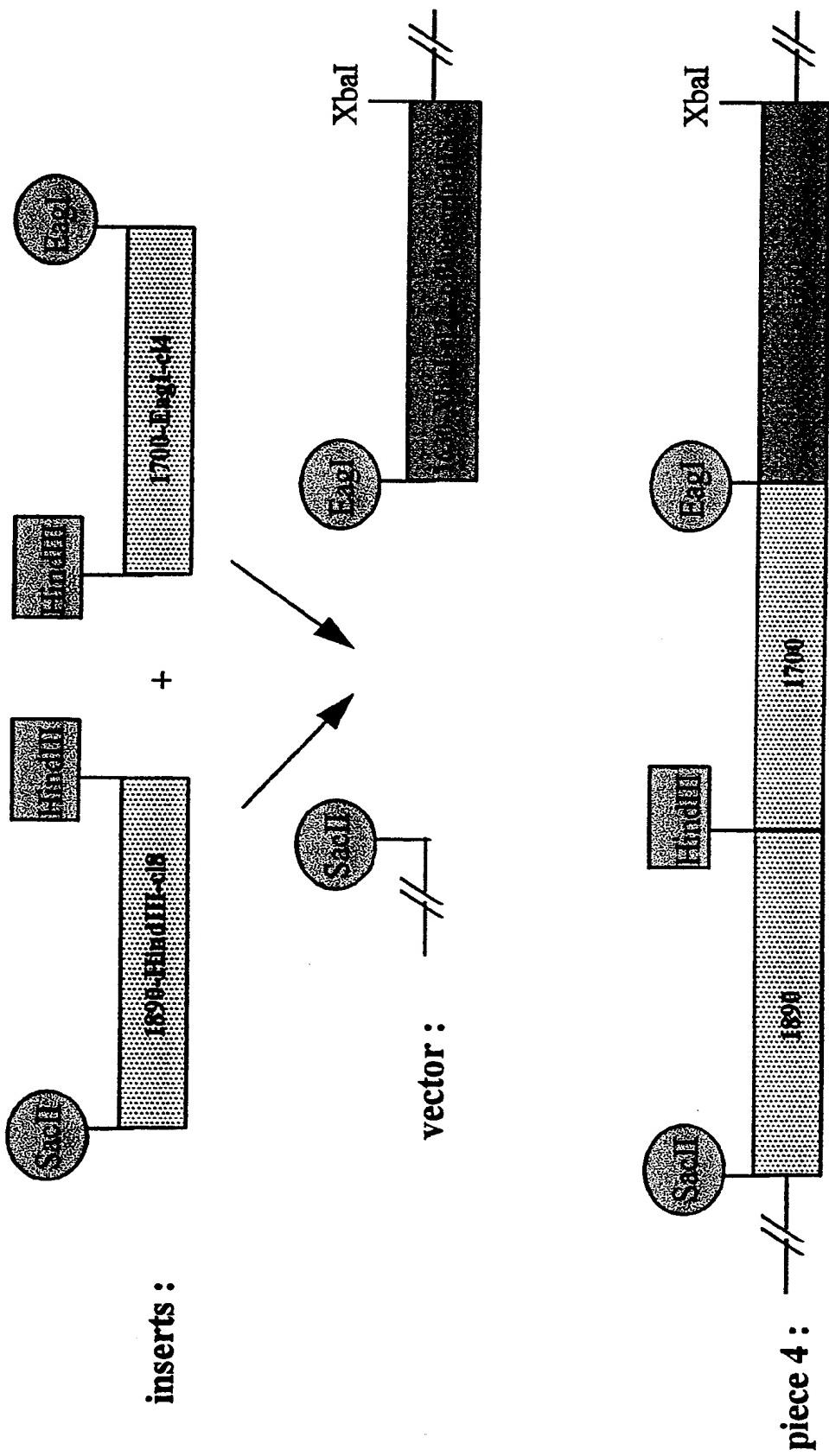


Fig. 15

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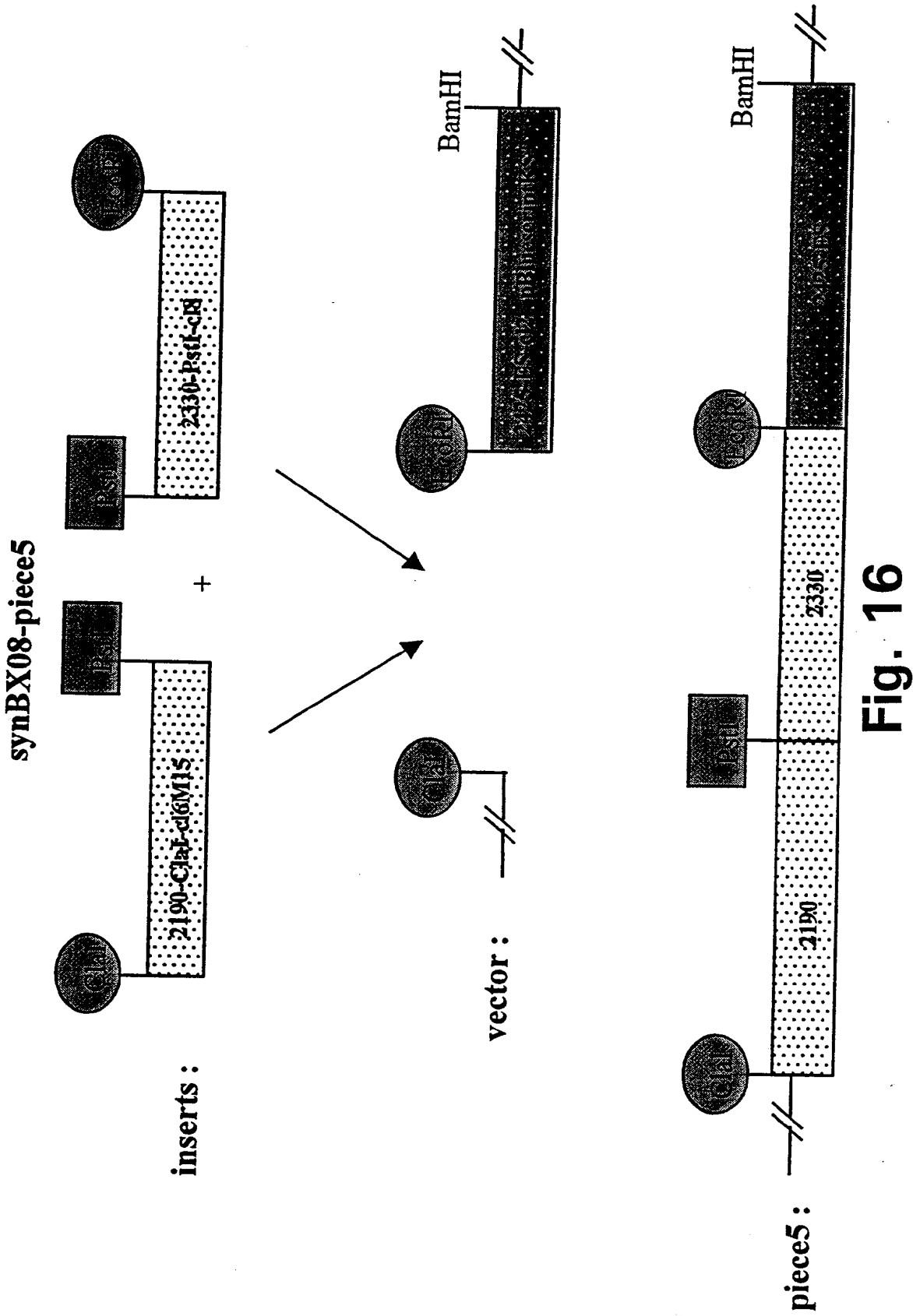


Fig. 16

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Figure 17 : synBX08-piece8gp160

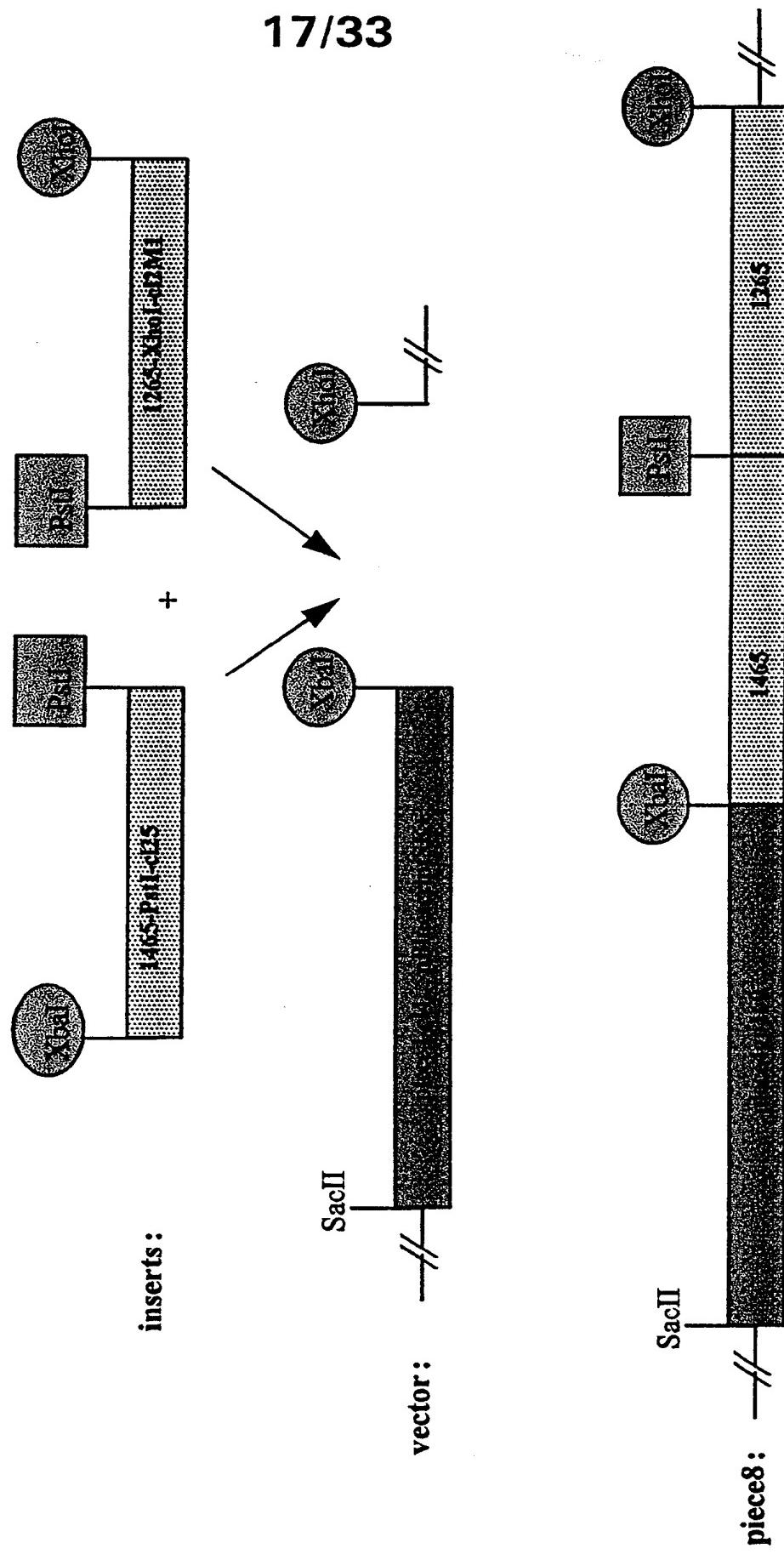
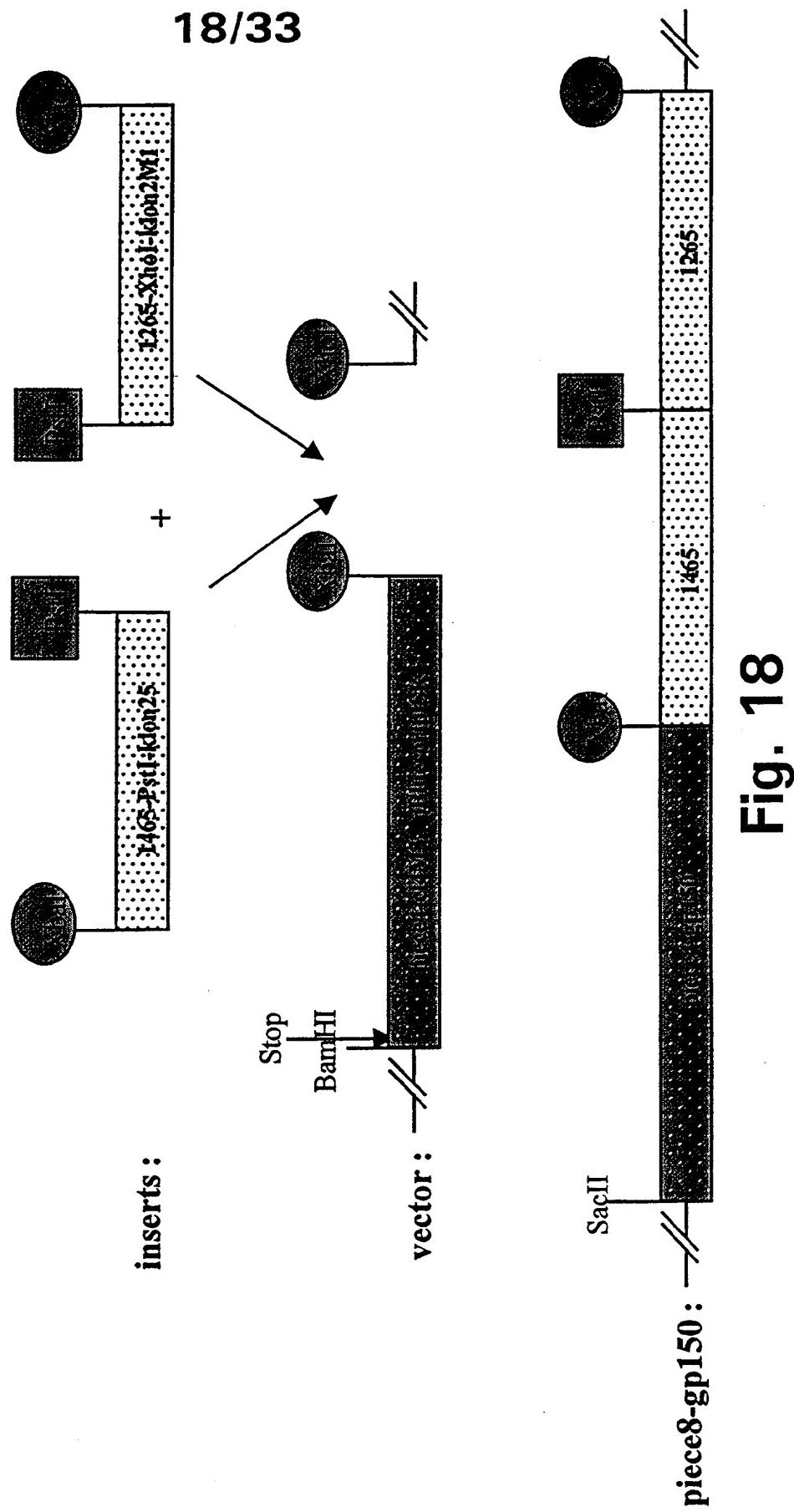


Fig. 17

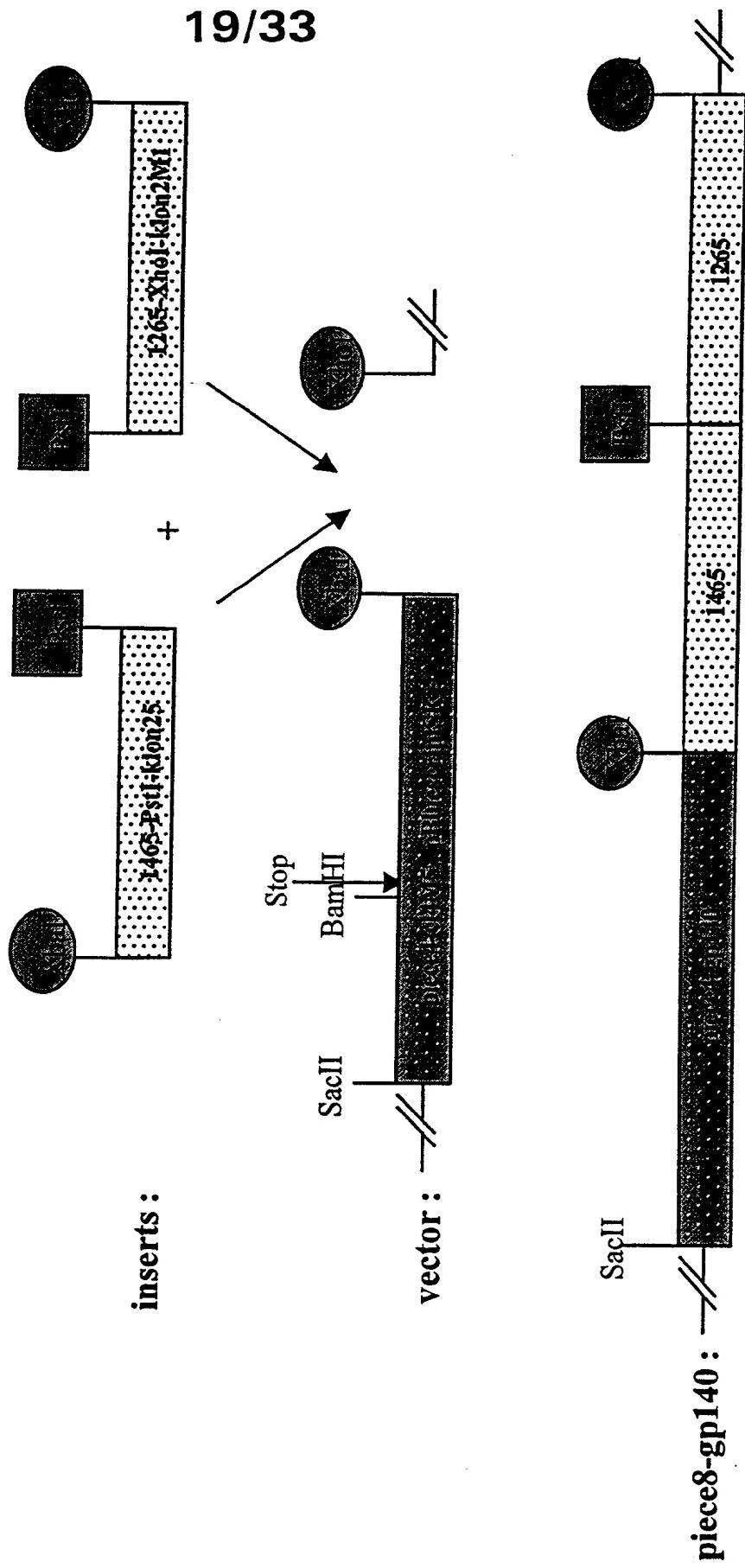
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synBX08-piece8-gp150

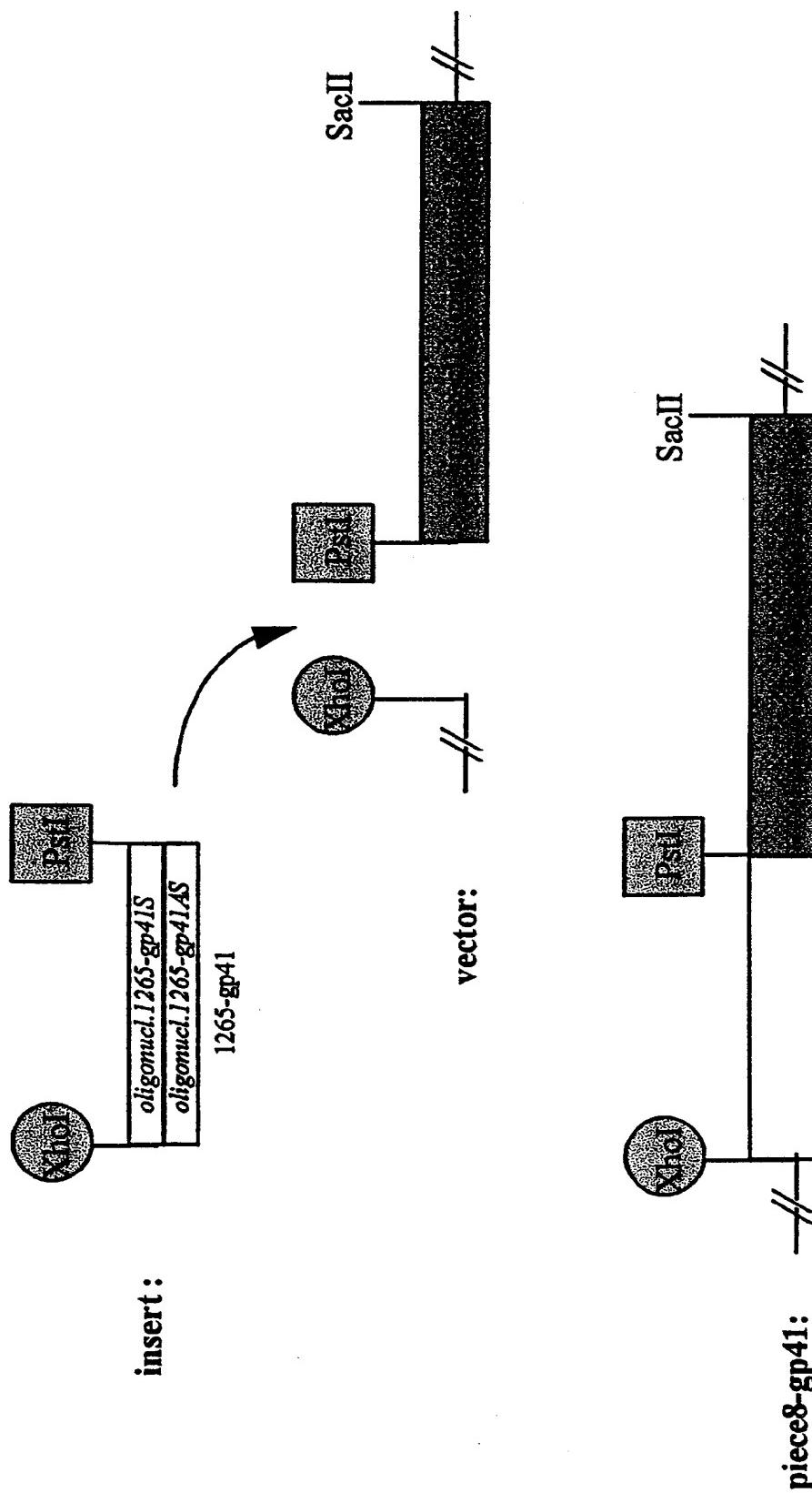
**Fig. 18**

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synBX08-piece8-gp140

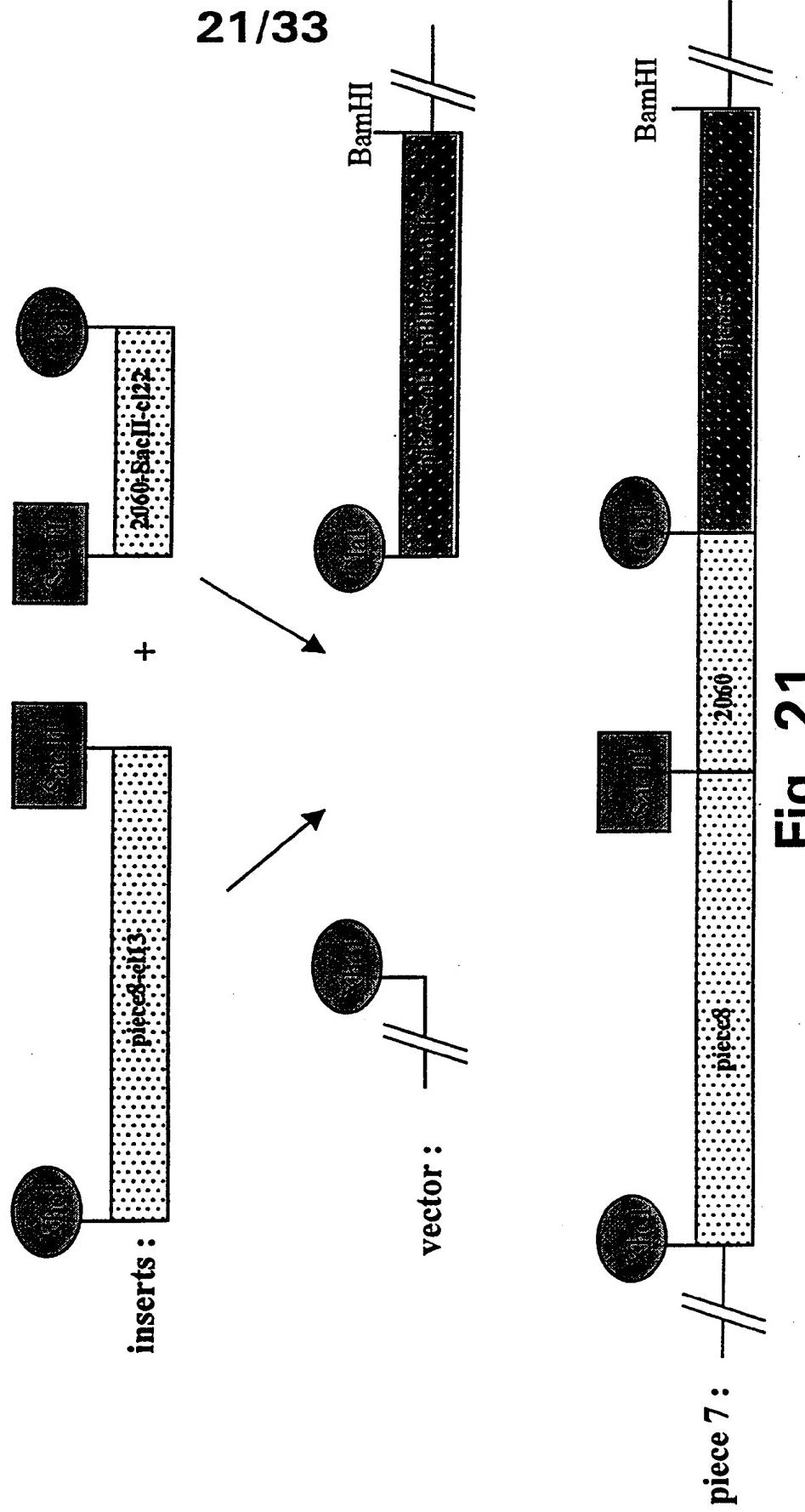
**Fig. 19**

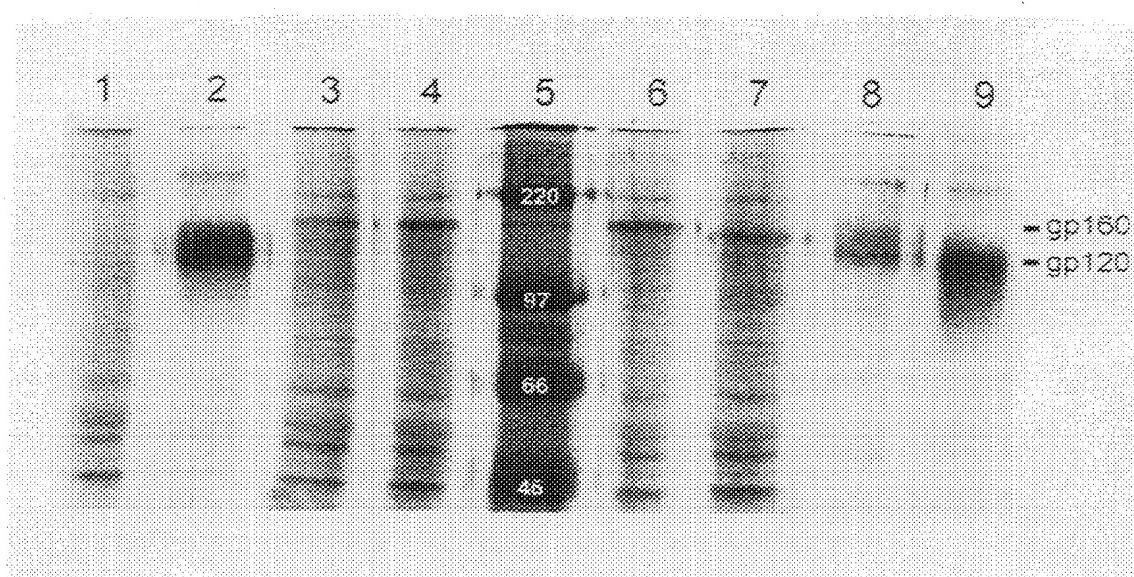
20/33

Figure 20: symBX08-piece8-gp41**Fig. 20**

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'synBX08-piece 7

**Fig. 21**

22/33**Fig. 22 A**

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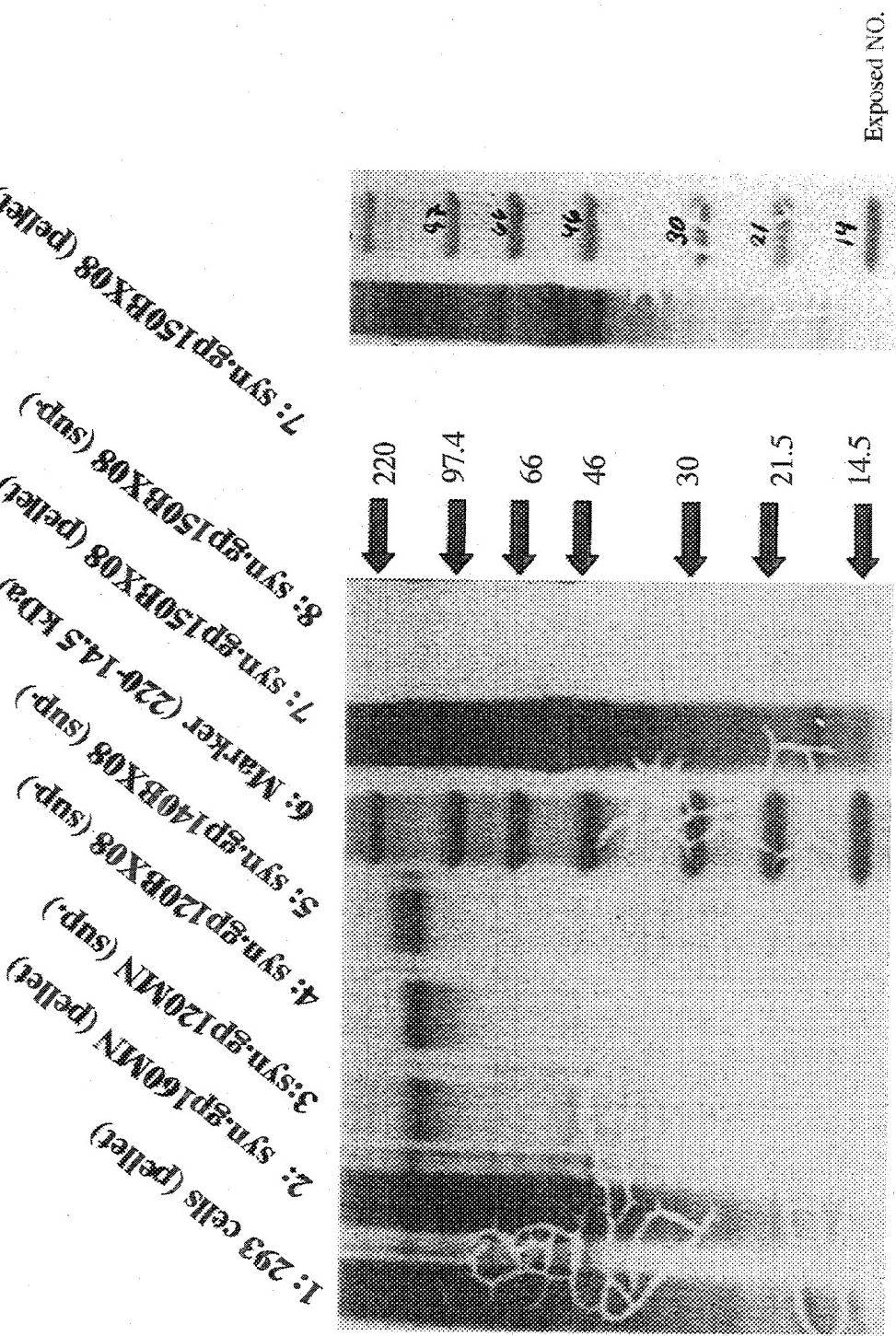


Fig. 22 B

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Panel A

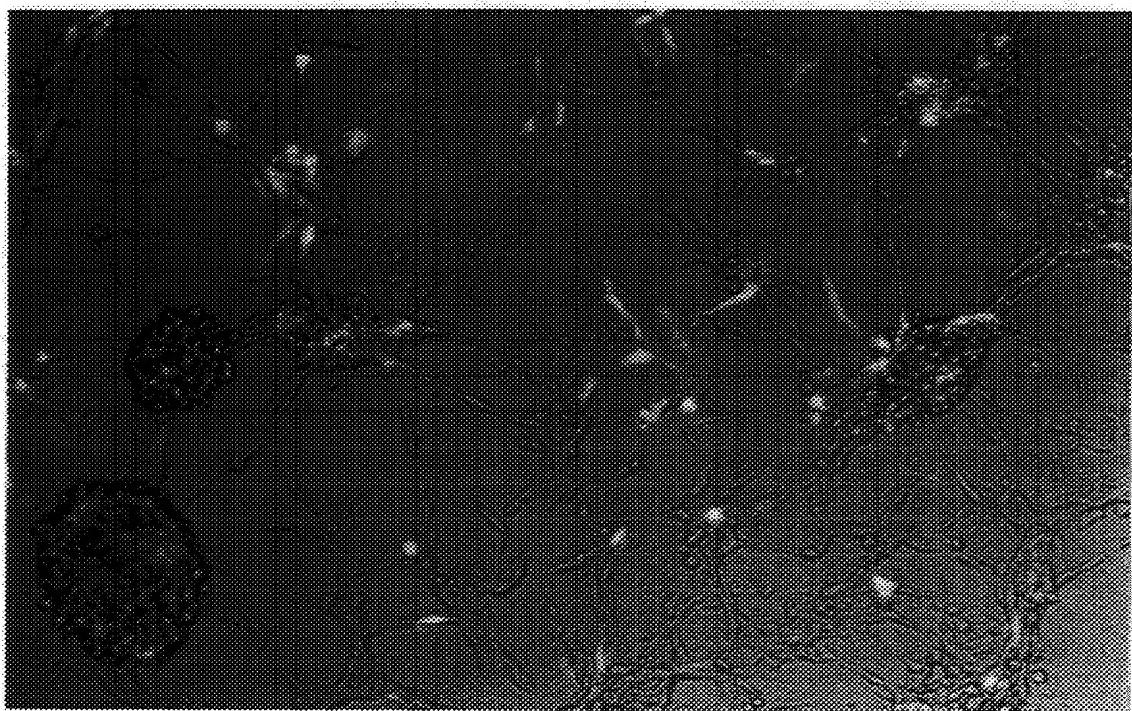


Fig. 22 C

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Panel B

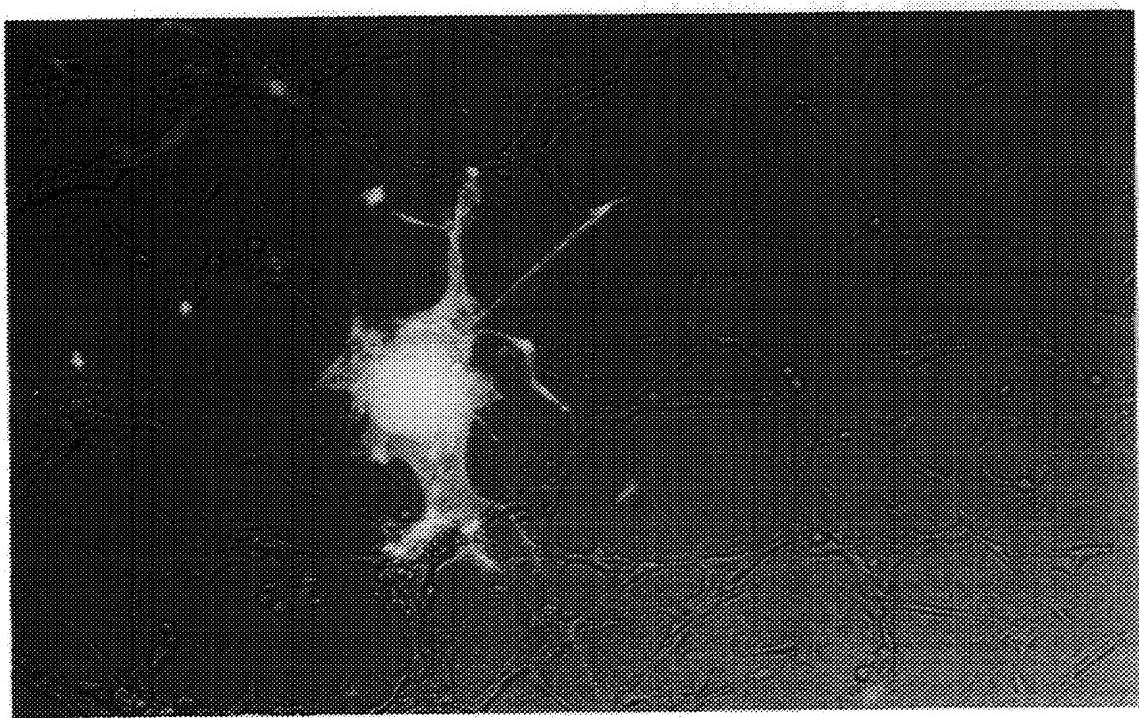


Fig. 22 C

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Panel C

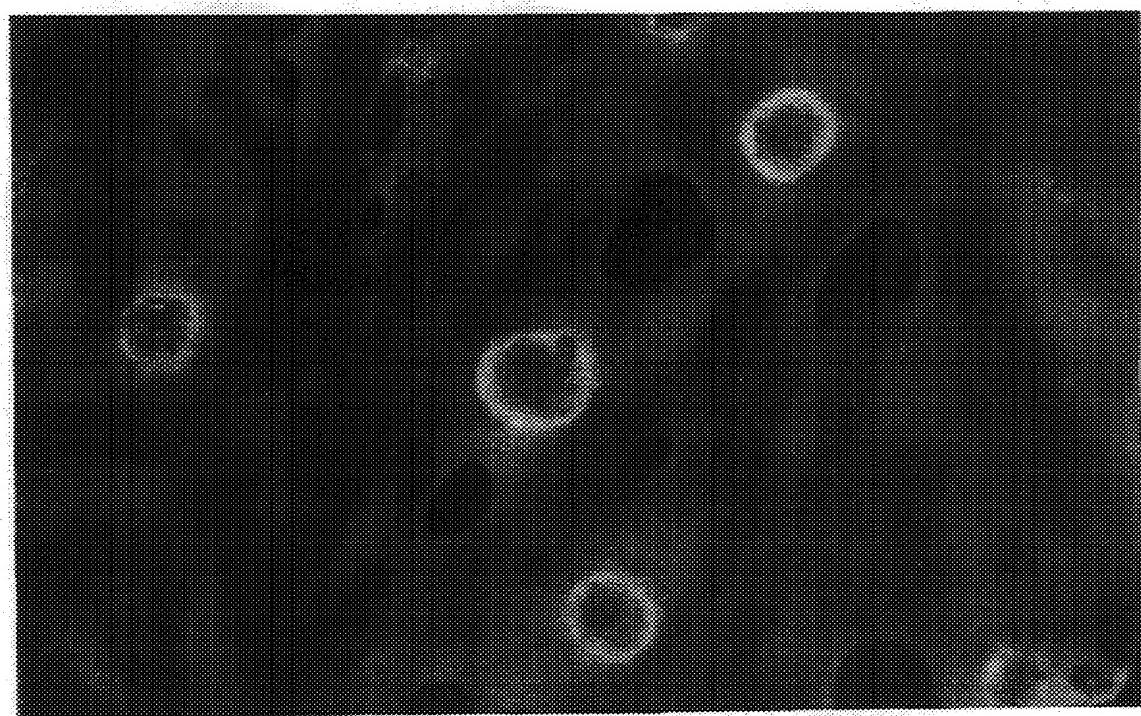


Fig. 22 C

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IgG1 anti-V3 BX08 from Balb/c mice DNA vaccinated with envelope genes in WRG7079

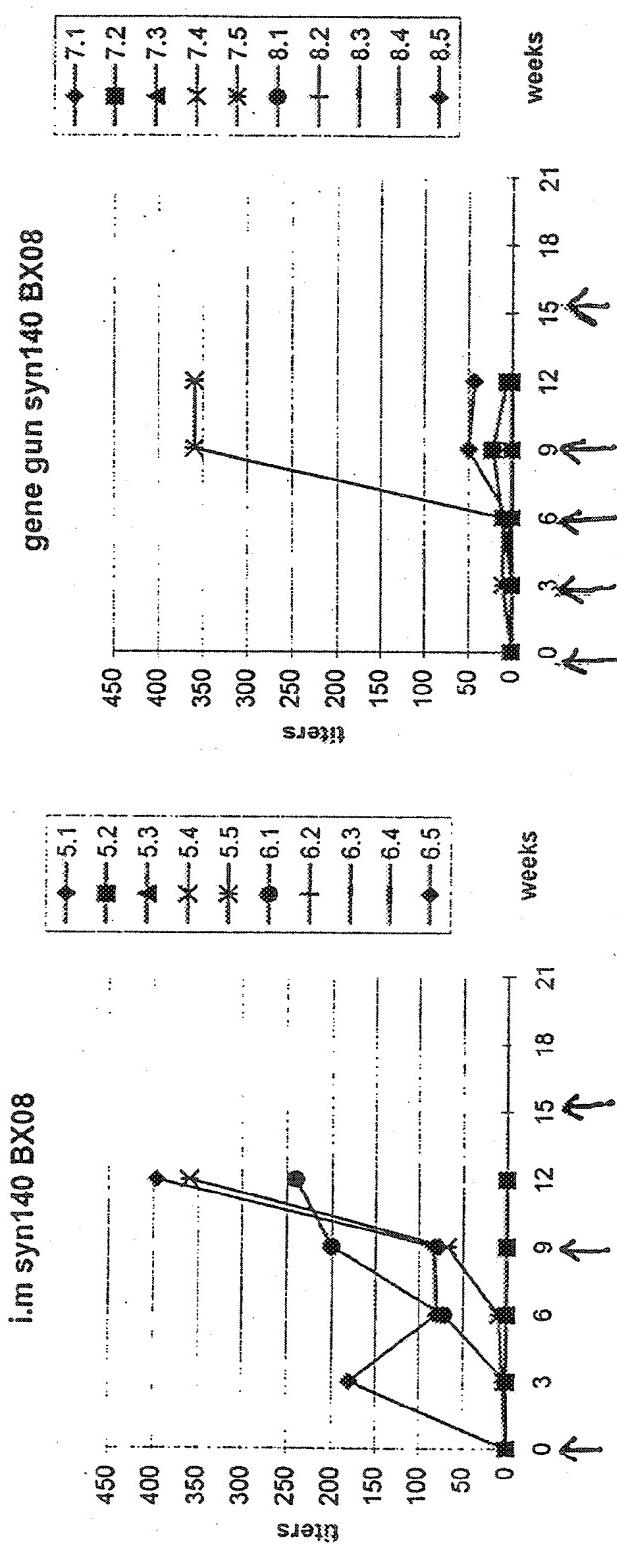


Fig. 23

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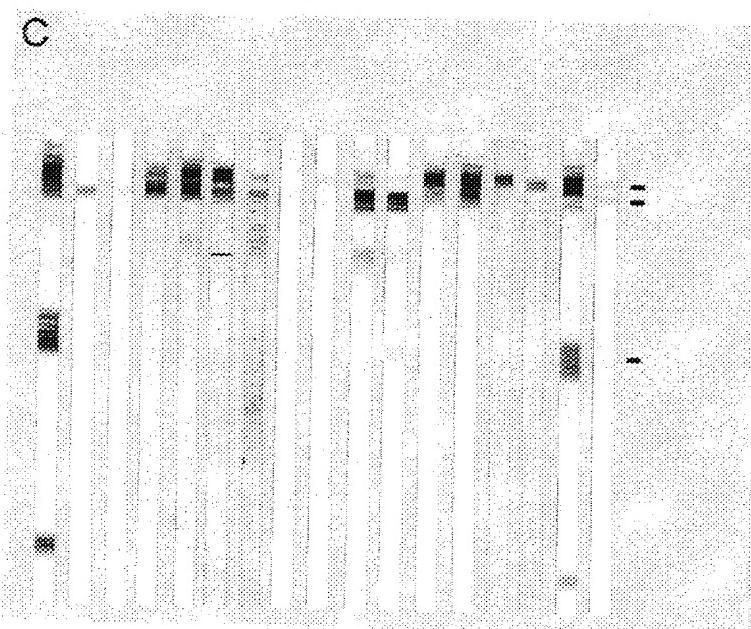


Fig. 24

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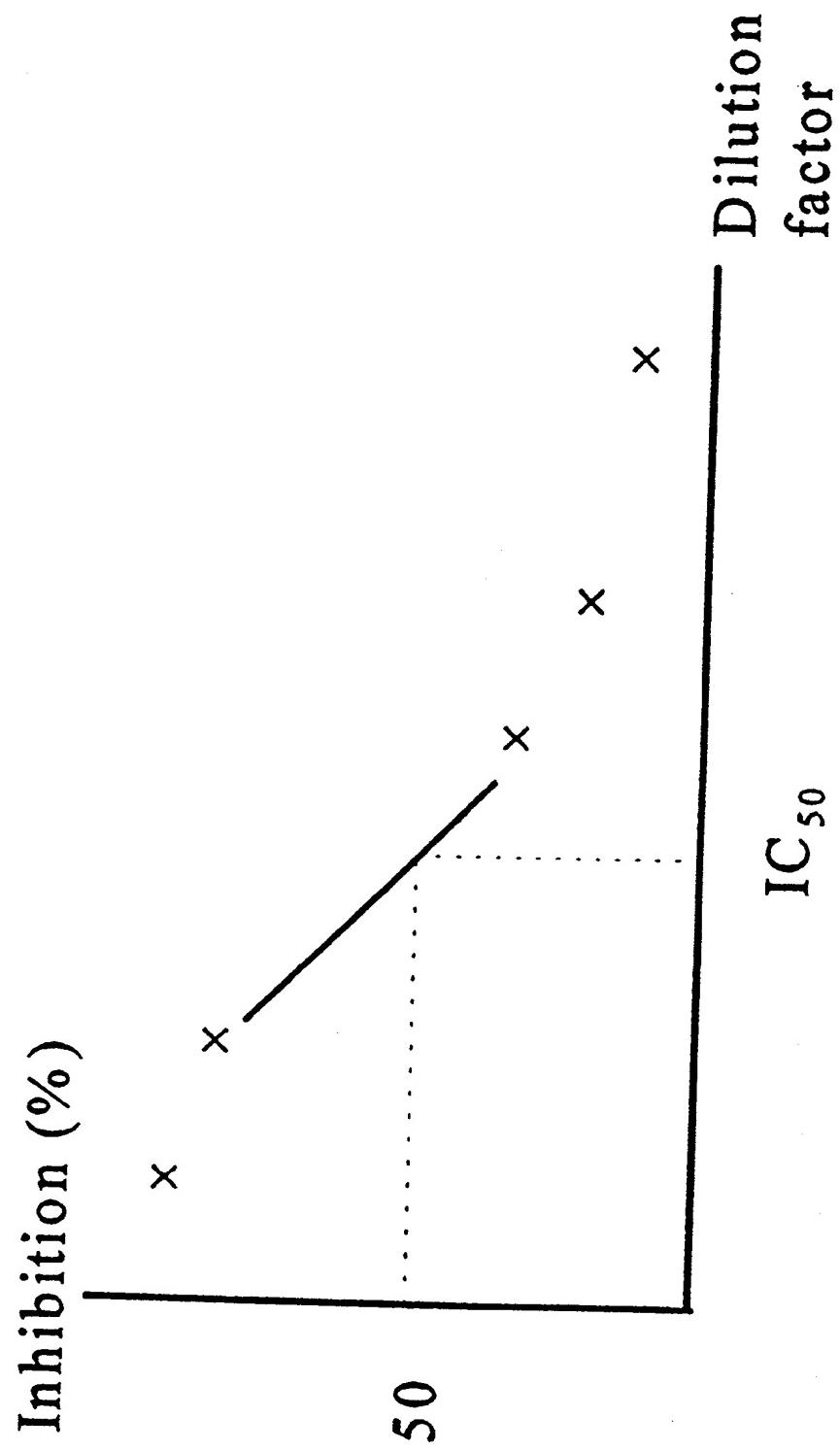


Fig. 25

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Fig. 26 A CTL activity induced by different synthetic DNA vaccines

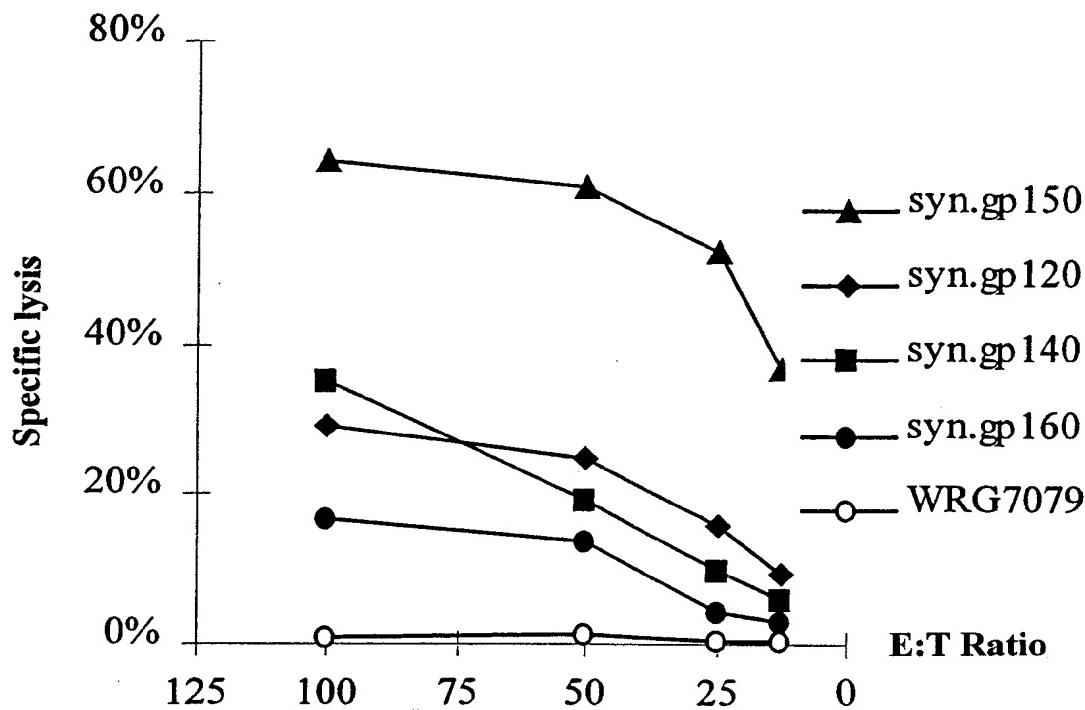
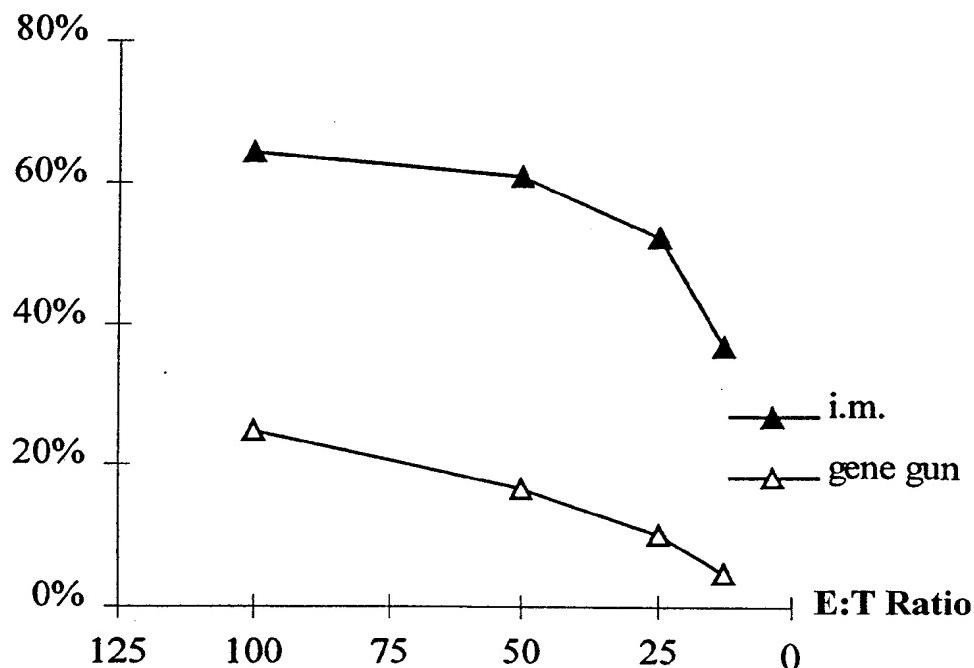


Fig. 26 B CTL activity induced by different DNA delivery methods.

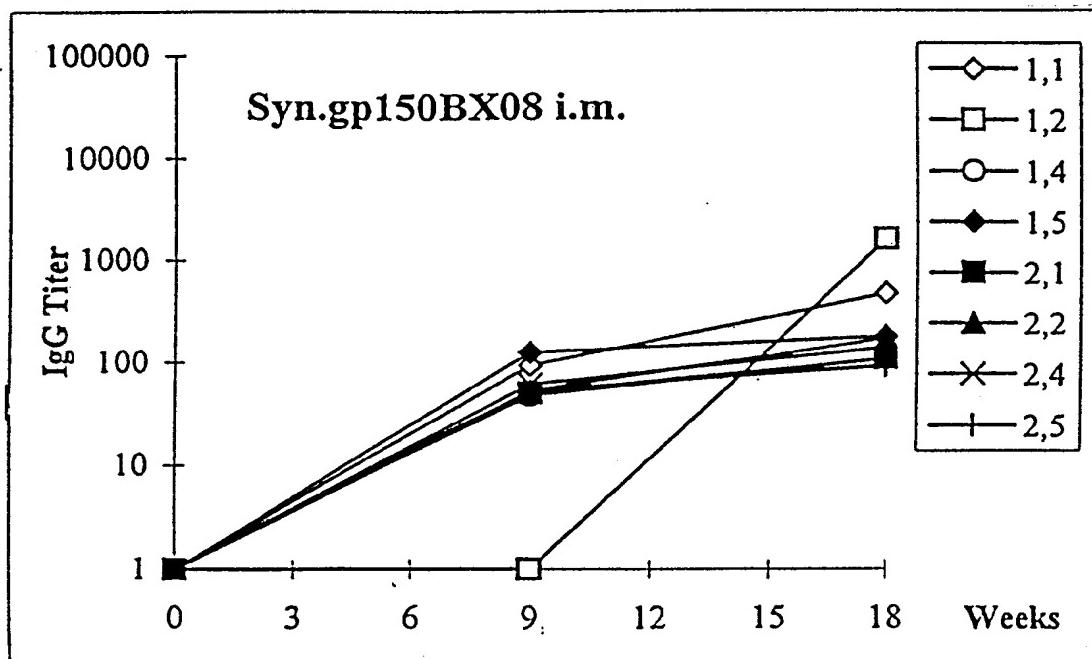
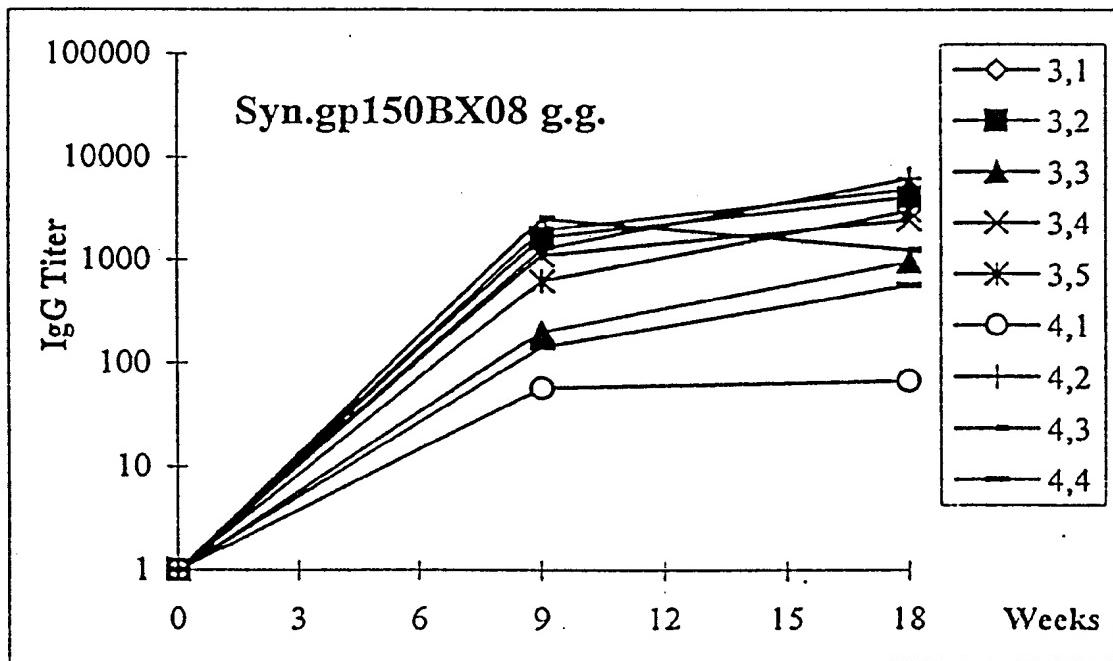
**Fig. 26**

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Percentage of
antiserum positive to
gp120_{IIIb} and gp41_{IIIb}
in western blot assay

Week		0	9	18
syn.gp120 _{BX08}	gp120	0	65	90
	gp41	0	0	35
syn.gp140 _{BX08}	gp120	0	65	100
	gp41	0	95	100
syn.gp150 _{BX08}	gp120	0	30	41
	gp41	0	41	53
syn.gp160 _{BX08}	gp120	0	32	50
	gp41	0	44	64
wt.gp160 _{BX08}	gp120	0	nd	53
	gp41	0	nd	48
wt.gp160 _{BX08} /pRev	gp120	0	nd	5
	gp41	0	nd	55

Fig. 27

32/33**Fig. 28 A****Fig. 28 B****Fig. 28**

33/33

Fig. 29 A

i.m. immunisation

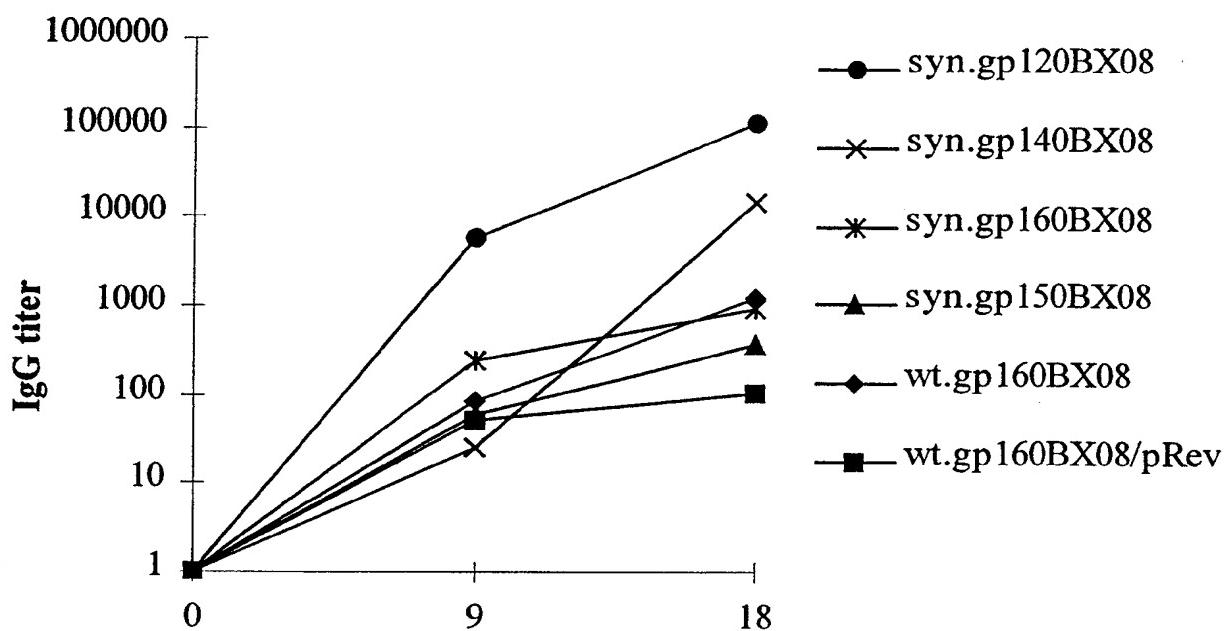


Fig. 29 B

gene gun immunisation

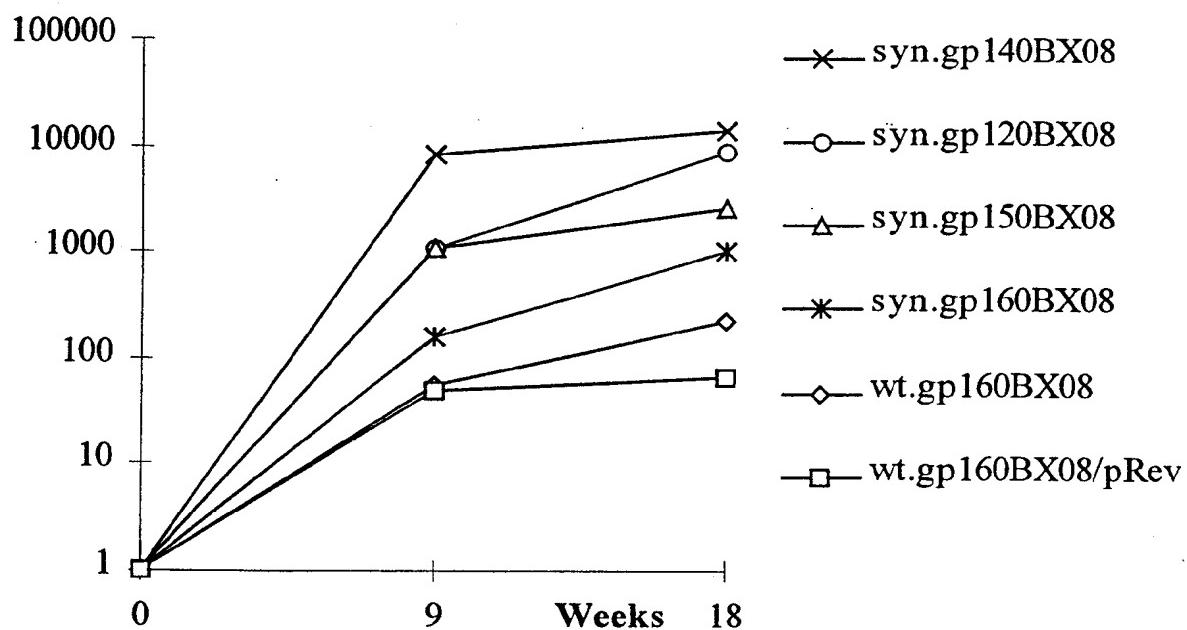


Fig. 29

SEQUENCE LISTING

<110> Statens Serum Institut

<120> Method for producing an nucleotide sequence construct with optimised codons for an HIV envelope based on a primary, clinical HIV isolate and the BX08 construct.

<130> 21924DK1

<160> 76

<170> FastSEQ for Windows Version 3.0

<210> 1

<211> 243

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (1)...(243)

<400> 1

gct	agc	gcg	gcc	gac	cgc	ctg	tgg	gtg	acc	gtg	tac	tac	ggc	gtg	ccc		48
Ala	Ser	Ala	Ala	Asp	Arg	Leu	Trp	Val	Thr	Val	Tyr	Tyr	Gly	Val	Pro		
1										10					15		

gtg	tgg	aag	gac	gcc	acc	acc	ctg	ttc	tgc	gcc	agc	gac	gcc	aag		96	
Val	Trp	Lys	Asp	Ala	Thr	Thr	Leu	Phe	Cys	Ala	Ser	Asp	Ala	Lys			
20										25					30		

gcc	tac	gac	acc	gag	gtg	cac	aac	gtg	tgg	gcc	acc	cac	gcg	tgc	gtg		144
Ala	Tyr	Asp	Thr	Glu	Val	His	Asn	Val	Trp	Ala	Thr	His	Ala	Cys	Val		
35										40					45		

ccc	acc	gac	ccc	aac	ccc	cag	gag	gtg	gtg	ctg	ggc	aac	gtg	acc	gag		192
Pro	Thr	Asp	Pro	Asn	Pro	Gln	Glu	Val	Val	Leu	Gly	Asn	Val	Thr	Glu		
50										55					60		

aac	ttc	aac	atg	ggc	aag	aac	aat	gtg	gag	cag	atg	cac	gag	gat		240	
Asn	Phe	Asn	Met	Gly	Lys	Asn	Asn	Met	Val	Glu	Gln	Met	His	Glu	Asp		
65										70					75		80

atc																243	
Ile																	

<210> 2
<211> 81
<212> PRT
<213> Artificial Sequence

<400> 2

Ala	Ser	Ala	Ala	Asp	Arg	Leu	Trp	Val	Thr	Val	Tyr	Tyr	Gly	Val	Pro		
1										5					10		15

Val Trp Lys Asp Ala Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
 20 25 30
 Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
 35 40 45
 Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
 50 55 60
 Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
 65 70 75 80
 Ile

<210> 3
 <211> 143
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(143)

<400> 3

gat atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg 48
 Asp Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu
 1 5 10 15

acc ccc ctg tgc gtg acc ctg aac tgc acc aag ctg aag aac agc acc 96
 Thr Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr
 20 25 30

gac acc aac aac acc cgc tgg ggc acc cag gag atg aag aac tgc ag 143
 Asp Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys
 35 40 45

<210> 4
 <211> 47
 <212> PRT
 <213> Artificial Sequence

<400> 4

Asp Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu
 1 5 10 15
 Thr Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr
 20 25 30
 Asp Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys
 35 40 45

<210> 5
 <211> 132
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(132)

<400> 5

ctg cag ctt caa cat cag cac cag cgt gcg caa caa gat gaa gca cga 48
 Leu Gln Leu Gln His Gln His Gln Arg Ala Gln Gln Asp Glu Ala Arg

1	5	10	15
gta cgc cct gtt cta cag cct gga cat cgt gcc cat cga caa cga caa Val Arg Pro Val Leu Gln Pro Gly His Arg Ala His Arg Gln Arg Gln	20	25	30
96			
cac cag cta ccg cct gcg cag ctg caa cac atc gat His Gln Leu Pro Pro Ala Gln Leu Gln His Ile Asp	35	40	132
<210> 6			
<211> 44			
<212> PRT			
<213> Artificial Sequence			
<400> 6			
leu Gln Leu Gln His Gln His Gln Arg Ala Gln Gln Asp Glu Ala Arg 1 5 10 15			
Val Arg Pro Val Leu Gln Pro Gly His Arg Ala His Arg Gln Arg Gln 20 25 30			
His Gln Leu Pro Pro Ala Gln Leu Gln His Ile Asp 35 40			
<210> 7			
<211> 161			
<212> DNA			
<213> Artificial Sequence			
<220>			
<221> CDS			
<222> (1) ... (161)			
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48			
cat cca ctt ctg cgc ccc cgc cgg ctt cgc cat cct gaa gtg caa caa His Pro Leu Leu Arg Pro Arg Arg Leu Arg His Pro Glu Val Gln Gln 20 25 30			
96			
caa gac ctt caa cgg cac cgg ccc ctg cac caa cgt gag cac cgt gca Gln Asp Leu Gln Arg His Arg Pro Leu His Gln Arg Glu His Arg Ala 35 40 45			
144			
tg cac cca cgg aat tc Val His Pro Arg Asn 50			
161			
<210> 8			
<211> 53			
<212> PRT			
<213> Artificial Sequence			
<400> 8			
le Asp His His Pro Gly Leu Pro Gln Gly Glu Leu Arg Ala His Pro 1 5 10 15			

His Pro Leu Leu Arg Pro Arg Arg Leu Arg His Pro Glu Val Gln Gln
 20 25 30
 Gln Asp Leu Gln Arg His Arg Pro Leu His Gln Arg Glu His Arg Ala
 35 40 45
 Val His Pro Arg Asn
 50

<210> 9
 <211> 254
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(254)

<400> 9

gaa ttc gcc ccg tgg tga gca ccc agc tgc tgc tga acg gca gca tgg	48
Glu Phe Ala Pro Trp * Ala Pro Ser Cys Cys * Thr Ala Ala Trp	
1 5 10	

ccg agg agg agg tgg tga tca gat ctg aga act tca cca aca acg cca	96
Pro Arg Arg Arg Trp * Ser Asp Leu Arg Thr Ser Pro Thr Thr Pro	
15 20 25	

aga cca tca tcg tgc agc tga acg aga gcg tgg aga tca act gca ccc	144
Arg Pro Ser Ser Cys Ser * Thr Arg Ala Trp Arg Ser Thr Ala Pro	
30 35 40	

gcc cca aca aca aca ccc gca aga gca tcc aca tcg gcc ctg gcc gcg	192
Ala Pro Thr Thr Pro Ala Arg Ala Ser Thr Ser Ala Leu Ala Ala	
45 50 55 60	

cct tct aca cca ccg gcg aca tca tcg gcg aca tcc gcc agg ccc act	240
Pro Ser Thr Pro Pro Ala Thr Ser Ser Ala Thr Ser Ala Arg Pro Thr	
65 70 75	

gca aca tct cta ga	254
Ala Thr Ser Leu	
80	

<210> 10
 <211> 80
 <212> PRT
 <213> Artificial Sequence

<400> 10

Glu Phe Ala Pro Trp Ala Pro Ser Cys Cys Thr Ala Ala Trp Pro Arg	
1 5 10 15	
Arg Arg Trp Ser Asp Leu Arg Thr Ser Pro Thr Thr Pro Arg Pro Ser	
20 25 30	
Ser Cys Ser Thr Arg Ala Trp Arg Ser Thr Ala Pro Ala Pro Thr Thr	
35 40 45	
Thr Pro Ala Arg Ala Ser Thr Ser Ala Leu Ala Ala Pro Ser Thr Pro	
50 55 60	
Pro Ala Thr Ser Ser Ala Thr Ser Ala Arg Pro Thr Ala Thr Ser Leu	
65 70 75 80	

<210> 11
<211> 92
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(92)

<400> 11
tct aga acc aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg 48
Ser Arg Thr Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu
 5 10 15

cgc gag aag ttc aac aac acc acc atc gtg ttc aac cag agc tc 92
Arg Glu Lys Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser
 20 25 30

<210> 12
<211> 30
<212> PRT
<213> Artificial Sequence

<400> 12
Ser Arg Thr Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu
 1 5 10 15
Arg Glu Lys Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser
 20 25 30

<210> 13
<211> 130
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(130)

<400> 13
gag ctc cg^g cg^g cga ccc cga gat cgt gat gca cag ctt caa ctg cg^g 48
Glu Leu Arg Arg Pro Arg Asp Arg Asp Ala Gln Leu Gln Leu Arg
 1 5 10 15

cg^g cga gtt ctt cta ctg caa cac cac cca gct gtt caa cag cac ctg 96
Arg Arg Val Leu Leu Gln His His Pro Ala Val Gln Gln His Leu
 20 25 30

gaa cga gac caa cag cga ggg caa cat cac tag t 130
Glu Arg Asp Gln Gln Arg Gly Gln His His *
 35 40

<210> 14
<211> 42
<212> PRT
<213> Artificial Sequence

<400> 14

Glu Leu Arg Arg Arg Pro Arg Asp Arg Asp Ala Gln Leu Gln Leu Arg
 1 5 10 15
 Arg Arg Val Leu Leu Leu Gln His His Pro Ala Val Gln Gln His Leu
 20 25 30
 Glu Arg Asp Gln Gln Arg Gly Gln His His
 35 40

<210> 15
 <211> 164
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(164)

<400> 15

act	agt	ggc	acc	atc	acc	ctg	ccc	tgc	cgc	atc	aag	cag	atc	atc	aac		48
Thr	Ser	Gly	Thr	Ile	Thr	Leu	Pro	Cys	Arg	Ile	Lys	Gln	Ile	Ile	Asn		
1				5						10				15			

atg tgg cag gag gtg ggc aag gcc atg tac gcc ccc ccc atc ggc ggc
 Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro Ile Gly Gly
 20 25 30

cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg acc cgc gac
 Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Thr Arg Asp
 35 40 45

ggc ggc agc gac aac tcg ag
 Gly Gly Ser Asp Asn Ser
 50

<210> 16
 <211> 54
 <212> PRT
 <213> Artificial Sequence

<400> 16

Thr	Ser	Gly	Thr	Ile	Thr	Leu	Pro	Cys	Arg	Ile	Lys	Gln	Ile	Ile	Asn		164
1				5						10				15			
Met	Trp	Gln	Glu	Val	Gly	Lys	Ala	Met	Tyr	Ala	Pro	Pro	Ile	Gly	Gly		
				20					25				30				
Gln	Ile	Lys	Cys	Leu	Ser	Asn	Ile	Thr	Gly	Leu	Leu	Leu	Thr	Arg	Asp		
				35				40					45				
Gly	Gly	Ser	Asp	Asn	Ser												
				50													

<210> 17
 <211> 200
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(200)

<400> 17

ctc gag cag cg_g caa gga gat ttt ccg ccc cgg cgg cgg cga cat gcg 48
 Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1 5 10 15

cga caa ctg gc_g cag cga gct gta caa gta caa ggt ggt gaa gat cga 96
 Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Glu Asp Arg
 20 25 30

gcc cct ggg cat cg_c ccc cac caa ggc caa gc_g ccg cgt ggt gca gc_g 144
 Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
 35 40 45

cga gaa gc_g cg_c cgt ggg cat cg_g cgc tat gtt cct ccg ctt cct ggg 192
 Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
 50 55 60

cgc tgc ag 200
 Arg Cys
 65

<210> 18
 <211> 66
 <212> PRT
 <213> Artificial Sequence

<400> 18
 Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1 5 10 15
 Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Glu Asp Arg
 20 25 30
 Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
 35 40 45
 Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
 50 55 60
 Arg Cys
 65

<210> 19
 <211> 212
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(212)

<400> 19
 ctc gag cag cg_g caa gga gat ttt ccg ccc cgg cgg cgg cga cat gcg 48
 Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1 5 10 15

cga caa ctg gc_g cag cga gct gta caa gta caa ggt ggt gaa gat cga 96
 Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Glu Asp Arg
 20 25 30

gcc cct ggg cat cg_c ccc cac caa ggc caa gc_g ccg cgt ggt gca gc_g 144
 Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
 35 40 45

cga gaa gcg cgc cta ggg cat cg_g cgc tat gtt cct cg_g ctt cct ggg 192
 Arg Glu Ala Arg Leu Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
 50 55 60

cgc tgc agc ccg ggg gat cc 212
 Arg Cys Ser Pro Gly Asp
 65 70

<210> 20
 <211> 70
 <212> PRT
 <213> Artificial Sequence

<400> 20
 Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg His Ala
 1 5 10 15
 Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
 20 25 30
 Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
 35 40 45
 Arg Glu Ala Arg Leu Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
 50 55 60
 Arg Cys Ser Pro Gly Asp
 65 70

<210> 21
 <211> 200
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(200)

<400> 21
 ctc gag ca_g cgg caa gga gat ttt cc_g ccc cgg cgg cgg cga cat gc_g 48
 Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg His Ala
 1 5 10 15

cga caa ctg gc_g ca_g gct gta caa gta caa ggt ggt gaa gat cga 96
 Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
 20 25 30

gcc cct ggg cat cgc ccc cac caa ggc caa gc_g cc_g cgt ggt gca gc_g 144
 Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
 35 40 45

cga gaa gag cgc cgt ggg cat cg_g cgc tat gtt cct cg_g ctt cct ggg 192
 Arg Glu Glu Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
 50 55 60

cgc tgc ag 200
 Arg Cys
 65

<210> 22

<211> 66
 <212> PRT
 <213> Artificial Sequence

<400> 22
 Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1 5 10 15
 Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
 20 25 30
 Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
 35 40 45
 Arg Glu Glu Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
 50 55 60
 Arg Cys
 65

<210> 23
 <211> 178
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(178)

<400> 23
 ctg cag gca gca cca tgg gcg ccg cca gcc tga ccc tga ccg tgc agg 48
 Leu Gln Ala Ala Pro Trp Ala Pro Pro Ala * Pro * Pro Cys Arg
 1 5 10
 ccc gcc agc tgc tga gcg gca tcg tgc agc agc aga aca acc tgc tgc 96
 Pro Ala Ser Cys * Ala Ala Ser Cys Ser Ser Arg Thr Thr Cys Cys
 15 20 25
 gcg cca tcg agg ccc agc agc acc tgc tcc agc tga ccg tgt ggg gca 144
 Ala Pro Ser Arg Pro Ser Ser Thr Cys Ser Ser * Pro Cys Gly Ala
 30 35 40
 tca agc agc tcc agg ccc gcg tgc tgg ctc tag a 178
 Ser Ser Ser Arg Pro Ala Cys Trp Leu *
 45 50

<210> 24
 <211> 54
 <212> PRT
 <213> Artificial Sequence

<400> 24
 Leu Gln Ala Ala Pro Trp Ala Pro Pro Ala Pro Pro Cys Arg Pro Ala
 1 5 10 15
 Ser Cys Ala Ala Ser Cys Ser Ser Arg Thr Thr Cys Cys Ala Pro Ser
 20 25 30
 Arg Pro Ser Ser Thr Cys Ser Ser Pro Cys Gly Ala Ser Ser Ser Ser
 35 40 45
 Arg Pro Ala Cys Trp Leu
 50

<210> 25

<211> 178
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(178)

<400> 25

ctg cag gca gca cca tgg gcg ccg cca gcc tga ccc tga ccg tgc agg	48		
Leu Gln Ala Ala Pro Trp Ala Pro Pro Ala * Pro * Pro Cys Arg			
1	5	10	

ccc gcc agc tgc tga gcg gca tcg tgc agc agc aga aca acc tgc tgc
 Pro Ala Ser Cys * Ala Ala Ser Cys Ser Ser Arg Thr Thr Cys Cys

15	20	25	96
----	----	----	----

gcg cca tcg agg ccc agc agc acc tgc tcc agc tga ccg tgt ggg gca
 Ala Pro Ser Arg Pro Ser Ser Thr Cys Ser Ser * Pro Cys Gly Ala

30	35	40	144
----	----	----	-----

tca agc agt gct gcg gcc gcg tgc tgg ctc tag a
 Ser Ser Ser Ala Ala Ala Cys Trp Leu *

45	50		178
----	----	--	-----

<210> 26
 <211> 54
 <212> PRT
 <213> Artificial Sequence

<400> 26

Leu Gln Ala Ala Pro Trp Ala Pro Pro Ala Pro Pro Cys Arg Pro Ala			
1	5	10	15
Ser Cys Ala Ala Ser Cys Ser Ser Arg Thr Thr Cys Cys Ala Pro Ser			
20		25	30
Arg Pro Ser Ser Thr Cys Ser Ser Pro Cys Gly Ala Ser Ser Ser Ala			
35		40	45
Ala Ala Ala Cys Trp Leu			
50			

<210> 27
 <211> 77
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(77)

<400> 27

tct aga gcg cta cct cca gga cca gcg ctt cct ggg cat gtg ggg ctg	48		
Ser Arg Ala Leu Pro Pro Gly Pro Ala Leu Pro Gly His Val Gly Leu			
1	5	10	15

ctc cgg caa gct gat ctg cac cac ggc cg
 Leu Arg Gln Ala Asp Leu His His Gly

20	25		77
----	----	--	----

<210> 28
<211> 25
<212> PRT
<213> Artificial Sequence

<400> 28
Ser Arg Ala Leu Pro Pro Gly Pro Ala Leu Pro Gly His Val Gly Leu
1 5 10 15
Leu Arg Gln Ala Asp Leu His His Gly
20 25

<210> 29
<211> 190
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1) ... (190)

<400> 29
cgg ccg tgc cct gga acg cca gct gga gca aca aga acc tga gcc aga 48
Arg Pro Cys Pro Gly Thr Pro Ala Gly Ala Thr Arg Thr * Ala Arg
1 5 10 15

ttt ggg aca aca tga cct gga tgg agt ggg agc gcg aga tca gca act 96
Phe Gly Thr Thr * Pro Gly Trp Ser Gly Ser Ala Arg Ser Ala Thr
20 25 30

aca ccg aga tca tct aca gcc tga tcg agg aga gcc aga acc agc agg 144
Thr Pro Arg Ser Ser Thr Ala * Ser Arg Arg Ala Arg Thr Ser Arg
35 40 45

aga aga acg agc tgg acc tgc tcc agc tgg aca agt ggg caa gct t 190
Arg Arg Thr Ser Trp Thr Cys Ser Ser Trp Thr Ser Gly Gln Ala
50 55 60

<210> 30
<211> 60
<212> PRT
<213> Artificial Sequence

<400> 30
Arg Pro Cys Pro Gly Thr Pro Ala Gly Ala Thr Arg Thr Ala Arg Phe
1 5 10 15
Gly Thr Thr Pro Gly Trp Ser Gly Ser Ala Arg Ser Ala Thr Thr Pro
20 25 30
Arg Ser Ser Thr Ala Ser Arg Arg Ala Arg Thr Ser Arg Arg Arg Thr
35 40 45
Ser Trp Thr Cys Ser Ser Trp Thr Ser Gly Gln Ala
50 55 60

<210> 31
<211> 177
<212> DNA
<213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(177)

<400> 31

aag ctt gtg gaa ctg gtt caa cat cac caa ctg gct gtg gta cat caa	48
Lys Leu Val Glu Leu Val Gln His His Gln Leu Ala Val Val His Gln	
1 5 10 15	

gat ttt cat cat gat cgt ggg cgg cct gat cgg cct gcg cat cgt gtt

Asp Phe His His Asp Arg Gly Arg Pro Asp Arg Pro Ala His Arg Val	96
20 25 30	

cac cgt gct gag cat cgt gaa ccg cgt gcg cca ggg cta cag ccc cct

His Arg Ala Glu His Arg Glu Pro Arg Ala Pro Gly Leu Gln Pro Pro	144
35 40 45	

gag ctt cca gac ccg cct gcc cgt gcc ccg cgg

Glu Leu Pro Asp Pro Pro Ala Arg Ala Pro Arg	177
50 55	

<210> 32
 <211> 59
 <212> PRT
 <213> Artificial Sequence

<400> 32

Lys Leu Val Glu Leu Val Gln His His Gln Leu Ala Val Val His Gln	
1 5 10 15	
Asp Phe His His Asp Arg Gly Arg Pro Asp Arg Pro Ala His Arg Val	
20 25 30	
His Arg Ala Glu His Arg Glu Pro Arg Ala Pro Gly Leu Gln Pro Pro	
35 40 45	
Glu Leu Pro Asp Pro Pro Ala Arg Ala Pro Arg	
50 55	

<210> 33
 <211> 140
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(140)

<400> 33

ccg cgg ccc cga ccg ccc cga ggg cat cga gga gga ggg cgg cga gcg	48
Pro Arg Pro Arg Pro Pro Arg Gly His Arg Gly Gly Arg Arg Ala	
1 5 10 15	

cga ccg cga ccg cag cac ccg cct ggt gac ccg ctt cct gcc cct gat

Arg Pro Arg Pro Gln His Pro Pro Gly Asp Arg Leu Pro Ala Pro Asp	96
20 25 30	

ctg gga cga cct gcg cag cct gtt cct gtt cag cta cca tcg at

Leu Gly Arg Pro Ala Gln Pro Val Pro Val Gln Leu Pro Ser	140
35 40 45	

<210> 34
<211> 46
<212> PRT
<213> Artificial Sequence

<400> 34
Pro Arg Pro Arg Pro Pro Arg Gly His Arg Gly Gly Gly Arg Arg Ala
1 5 10 15
Arg Pro Arg Pro Gln His Pro Pro Gly Asp Arg Leu Pro Ala Pro Asp
20 25 30
Leu Gly Arg Pro Ala Gln Pro Val Pro Val Gln Leu Pro Ser
35 40 45

<210> 35
<211> 129
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(129)

<400> 35
atc gat tgc gcg acc tgc tgc tga tcg tgg ccc gca tcg tgg agc tgc 48
Ile Asp Cys Ala Thr Cys Cys * Ser Trp Pro Ala Ser Trp Ser Cys
1 5 10 15

tgg gcc ggc gcg gct ggg aga tcc tga agt act ggt gga acc tgc tcc 96
Trp Ala Gly Ala Ala Gly Arg Ser * Ser Thr Gly Gly Thr Cys Ser
20 25 30

agt act gga gcc agg agc tga aga act ctg cag 129
Ser Thr Gly Ala Arg Ser * Arg Thr Leu Gln
35 40

<210> 36
<211> 40
<212> PRT
<213> Artificial Sequence

<400> 36
Ile Asp Cys Ala Thr Cys Cys Ser Trp Pro Ala Ser Trp Ser Cys Trp
1 5 10 15
Ala Gly Ala Ala Gly Arg Ser Ser Thr Gly Gly Thr Cys Ser Ser Thr
20 25 30
Gly Ala Arg Ser Arg Thr Leu Gln
35 40

<210> 37
<211> 114
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(114)

<400> 37
ctg cag tga gcc tgc tga acg cca ccg cca tcg ccg tgg ccg agg gca 48
Leu Gln * Ala Cys * Thr Pro Pro Pro Ser Pro Trp Pro Arg Ala
1 5 10

ccg acc gcg tga tcg agg tgg tgc agc gca tct ggc gcg gca tcc tgc 96
Pro Thr Ala * Ser Arg Trp Cys Ser Ala Ser Gly Ala Ala Ser Cys
15 20 25

aca tcc cca ccc gaa ttc 114
Thr Ser Pro Pro Glu Phe
30 35

<210> 38
<211> 35
<212> PRT
<213> Artificial Sequence

<400> 38
Leu Gln Ala Cys Thr Pro Pro Pro Ser Pro Trp Pro Arg Ala Pro Thr 41
1 5 10 15
Ala Ser Arg Trp Cys Ser Ala Ser Gly Ala Ala Ser Cys Thr Ser Pro
20 25 30

Pro Glu Phe
35

<210> 39
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(41)

<400> 39
gaa ttc gcc agg gct tcg agc gcg ccc tgc tgt aag gat cc 41
Glu Phe Ala Arg Ala Ser Ser Ala Pro Cys Cys Lys Asp
1 5 10

<210> 40
<211> 13
<212> PRT
<213> Artificial Sequence

<400> 40
Glu Phe Ala Arg Ala Ser Ser Ala Pro Cys Cys Lys Asp
1 5 10

<210> 41
<211> 506
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(506)

<400> 41
 gct agc gcg gcc gac cgc ctg tgg gtg acc gtg tac tac ggc gtg ccc 48
 Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1 5 10 15

gtg tgg aag gac gcc acc acc ctg ttc tgc gcc agc gac gcc aag 96
 Val Trp Lys Asp Ala Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
 20 25 30

gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg 144
 Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
 35 40 45

ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag 192
 Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
 50 55 60

aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat 240
 Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
 65 70 75 80

atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg acc 288
 Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
 85 90 95

ccc ctg tgc gtg acc ctg aac tgc acc aag ctg aag aac agc acc gac 336
 Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
 100 105 110

acc aac aac acc cgc tgg ggc acc cag gag atg aag aac tgc agc ttc 384
 Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
 115 120 125

aac atc agc acc agc gtg cgc aac aag atg aag cgc gag tac gcc ctg 432
 Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
 130 135 140

ttc tac agc ctg gac atc gtg ccc atc gac aac gac aac acc agc tac 480
 Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
 145 150 155 160

cgc ctg cgc agc tgc aac aca tcg at 506
 Arg Leu Arg Ser Cys Asn Thr Ser
 165

<210> 42
<211> 168
<212> PRT
<213> Artificial Sequence

<400> 42
 Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1 5 10 15
 Val Trp Lys Asp Ala Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
 20 25 30
 Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
 35 40 45

Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
 50 55 60
 Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
 65 70 75 80
 Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
 85 90 95
 Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
 100 105 110
 Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
 115 120 125
 Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
 130 135 140
 Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
 145 150 155 160
 Arg Leu Arg Ser Cys Asn Thr Ser
 165

<210> 43
<211> 374
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(374)

<400> 43

tct aga acc aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg	48
Ser Arg Thr Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu	
1 5 10 15	

cgc gag aag ttc aac aac acc acc atc gtg ttc aac cag cag tcc ggc	96
Arg Glu Lys Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly	
20 25 30	

ggc gac ccc gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc	144
Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe	
35 40 45	

ttc tac tgc aac acc acc cag ctg ttc aac agc acc tgg aac gag acc	192
Phe Tyr Cys Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr	
50 55 60	

aac agc gag ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc	240
Asn Ser Glu Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg	
65 70 75 80	

atc aag cag atc atc aac atg tgg cag gag gtg ggc aag gcc atg tac	288
Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr	
85 90 95	

gcc ccc ccc atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc	336
Ala Pro Pro Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly	
100 105 110	

ctg ctg ctg acc cgc gac ggc ggc agc gac aac tcg ag	374
Leu Leu Leu Thr Arg Asp Gly Gly Ser Asp Asn Ser	
115 120	

<210> 44
 <211> 124
 <212> PRT
 <213> Artificial Sequence

<400> 44
 Ser Arg Thr Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu
 1 5 10 15
 Arg Glu Lys Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly
 20 25 30
 Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe
 35 40 45
 Phe Tyr Cys Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr
 50 55 60
 Asn Ser Glu Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg
 65 70 75 80
 Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr
 85 90 95
 Ala Pro Pro Ile Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly
 100 105 110
 Leu Leu Leu Thr Arg Asp Gly Gly Ser Asp Asn Ser
 115 120

<210> 45
 <211> 1277
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(1277)

<400> 45
 gct agc gcg gcc gac cgc ctg tgg gtg acc gtg tac tac ggc gtg ccc 48
 Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1 5 10 15
 gtg tgg aag gac gcc acc acc ctg ttc tgc gcc agc gac gcc aag 96
 Val Trp Lys Asp Ala Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
 20 25 30
 gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg 144
 Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
 35 40 45
 ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag 192
 Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
 50 55 60
 aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat 240
 Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
 65 70 75 80
 atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg acc 288
 Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
 85 90 95
 ccc ctg tgc gtg acc ctg aac tgc acc aag ctg aag aac agc acc gac 336

Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp			
100	105	110	
acc aac aac acc cgc tgg ggc acc cag gag atg aag aac tgc agc ttc			384
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe			
115	120	125	
aac atc agc acc agc gtg cgc aac aag atg aag cgc gag tac gcc ctg			432
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu			
130	135	140	
ttc tac agc ctg gac atc gtg ccc atc gac aac gac aac acc agc tac			480
Phe Tyr Ser Leu Asp Ile Val Pro Ile-Asp Asn Asp Asn Thr Ser Tyr			
145	150	155	160
cgc ctg cgc agc tgc aac aca tcg atc atc acc cag gcc tgc ccc aag			528
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys			
165	170	175	
gtg agc ttc gag ccc atc ccc atc cac ttc tgc gcc ccc gcc ggc ttc			576
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe			
180	185	190	
gcc atc ctg aag tgc aac aac aag acc ttc aac ggc acc ggc ccc tgc			624
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys			
195	200	205	
acc aac gtg agc acc gtg cag tgc acc cac gga att cgc ccc gtg gtg			672
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val			
210	215	220	
agc acc cag ctg ctg aac ggc agc ctg gcc gag gag gag gtg gtg			720
Ser Thr Gln Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val			
225	230	235	240
atc aga tct gag aac ttc acc aac gcc aag acc atc atc gtg cag			768
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln			
245	250	255	
ctg aac gag agc gtg gag atc aac tgc acc cgc ccc aac aac aac acc			816
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr			
260	265	270	
cgc aag agc atc cac atc ggc cct ggc cgc gcc ttc tac acc acc ggc			864
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Gly			
275	280	285	
gac atc atc ggc gac atc cgc cag gcc cac tgc aac atc tct aga acc			912
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr			
290	295	300	
aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg cgc gag aag			960
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys			
305	310	315	320
ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc ggc gac ccc			1008
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro			
325	330	335	

gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc ttc tac tgc Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys 340 345 350	1056
aac acc acc cag ctg ttc aac agc acc tgg aac gag acc aac agc gag Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu 355 360 365	1104
ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln 370 375 380	1152
att atc aac atg tgg cag gag gtg ggt-aag gcc atg tac gcc ccc ccc Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro 385 390 395 400	1200
atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu 405 410 415	1248
acc cgc gac ggc ggc agc gac aac tcg ag Thr Arg Asp Gly Gly Ser Asp Asn Ser 420 425	1277
<210> 46	
<211> 425	
<212> PRT	
<213> Artificial Sequence	
<400> 46	
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro 1 5 10 15	
Val Trp Lys Asp Ala Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys 20 25 30	
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val 35 40 45	
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu 50 55 60	
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp 65 70 75 80	
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr 85 90 95	
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp 100 105 110	
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe 115 120 125	
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu 130 135 140	
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr 145 150 155 160	
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys 165 170 175	
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe 180 185 190	
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys 195 200 205	
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val 210 215 220	

Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
 225 230 235 240
 Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
 245 250 255
 Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
 260 265 270
 Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
 275 280 285
 Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
 290 295 300
 Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
 305 310 315 320
 Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
 325 330 335
 Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
 340 345 350
 Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
 355 360 365
 Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
 370 375 380
 Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
 385 390 395 400
 Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
 405 410 415
 Thr Arg Asp Gly Gly Ser Asp Asn Ser
 420 425

<210> 47

<211> 1277

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (1)...(1277)

<400> 47

gct agc gcg gcc gac cgc ctg tgg gtg acc gtg tac tac ggc gtg ccc
 Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1 5 10 15

48

gtg tgg aag gac gcc acc acc ctg ttc tgc gcc agc gac gcc aag
 Val Trp Lys Asp Ala Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
 20 25 30

96

gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg
 Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
 35 40 45

144

ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag
 Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
 50 55 60

192

aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat
 Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
 65 70 75 80

240

atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg acc
 Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr

288

85	90	95	
ccc ctg tgc gtg acc ctg caa tgc acc aag ctg aag cag cag agc acc gac Pro Leu Cys Val Thr Leu Gln Cys Thr Lys Leu Lys Gln Ser Thr Asp 100	105	110	336
acc cag aac acc cgc tgg ggc acc cag gag atg aag aac tgc agc ttc Thr Gln Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe 115	120	125	384
aac atc agc acc agc gtg cgc aac aag atg aag cgc gag tac gcc ctg Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu 130	135	140	432
ttc tac agc ctg gac atc gtg ccc atc gac aac gac aac acc agc tac Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr 145	150	155	480
cgc ctg cgc agc tgc aac aca tcg atc atc acc cag gcc tgc ccc aag Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys 165	170	175	528
gtg agc ttc gag ccc atc ccc atc cac ttc tgc gcc ccc ggc ttc Val Ser Phe Glu Pro Ile Pro His Phe Cys Ala Pro Ala Gly Phe 180	185	190	576
gcc atc ctg aag tgc aac aac aag acc ttc aac ggc acc ggc ccc tgc Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys 195	200	205	624
acc aac gtg agc acc gtg cag tgc acc cac gga att cgc ccc gtg gtg Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val 210	215	220	672
agc acc cag ctg ctg aac ggc agc ctg gcc gag gag gag gtg gtg Ser Thr Gln Leu Leu Asn Gly Ser Leu Ala Glu Glu Val Val 225	230	235	720
atc aga tct gag aac ttc acc aac aac gcc aag acc atc atc gtg cag Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln 245	250	255	768
ctg aac gag agc gtg gag atc aac tgc acc cgc ccc aac aac aac acc Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr 260	265	270	816
cgc aag agc atc cac atc ggc cct ggc cgc gcc ttc tac acc acc ggc Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly 275	280	285	864
gac atc atc ggc gac atc cgc cag gcc cac tgc aac atc tct aga acc Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr 290	295	300	912
aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg cgc gag aag Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys 305	310	315	960
ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc ggc gac ccc			1008

Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro			
325	330	335	
gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc ttc tac tgc			1056
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys			
340	345	350	
aac acc acc cag ctg ttc aac agc acc tgg aac gag acc aac agc gag			1104
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu			
355	360	365	
ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag			1152
Tyr Asn Ile Thr Ser Gly Thr Ile Thr-Leu Pro Cys Arg Ile Lys Gln			
370	375	380	
atc atc aac atg tgg cag gag gtg ggc aag gcc atg tac gcc ccc ccc			1200
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro			
385	390	395	400
atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg			1248
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu			
405	410	415	
acc cgc gac ggc ggc agc gac aac tcg ag			1277
Thr Arg Asp Gly Gly Ser Asp Asn Ser			
420	425		
<210> 48			
<211> 425			
<212> PRT			
<213> Artificial Sequence			
<400> 48			
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro			
1	5	10	15
Val Trp Lys Asp Ala Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys			
20	25	30	
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val			
35	40	45	
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu			
50	55	60	
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp			
65	70	75	80
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr			
85	90	95	
Pro Leu Cys Val Thr Leu Gln Cys Thr Lys Leu Lys Gln Ser Thr Asp			
100	105	110	
Thr Gln Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe			
115	120	125	
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu			
130	135	140	
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr			
145	150	155	160
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys			
165	170	175	
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe			
180	185	190	
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys			

195	200	205
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val		
210	215	220
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val		
225	230	235
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln		
245	250	255
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr		
260	265	270
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly		
275	280	285
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr		
290	295	300
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys		
305	310	315
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro		
325	330	335
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys		
340	345	350
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu		
355	360	365
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln		
370	375	380
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro		
385	390	395
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu		
405	410	415
Thr Arg Asp Gly Gly Ser Asp Asn Ser		
420	425	

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<210> 49  
<211> 144  
<212> PRT  
<213> Artificial Sequence
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<400> 49

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gtg	tgg	aag	gac	gcc	acc	acc	acc	ctg	ttc	tgc	gcc	agc	gac	gcc	aag	96
gcc	tac	gac	acc	gag	gtg	cac	aac	gtg	tgg	gcc	acc	cac	gcg	tgc	gtg	144
ccc	acc	gac	ccc	aac	ccc	cag	gag	gtg	gtg	ctg	ggc	aac	gtg	acc	gag	192
aat	ttc	aac	atg	ggc	aag	aac	aac	atg	gtg	gag	cag	atg	cac	gag	gat	240
atc	atc	agc	ctg	tgg	gac	cag	agc	ctg	aag	ccc	tgc	gtg	aag	ctg	acc	288
ccc	ctg	tgc	gtg	acc	ctg	caa	tgc	acc	aag	ctg	aag	cag	agc	acc	gac	336
acc	cag	aac	acc	cgc	tgg	ggc	acc	cag	gag	atg	aag	aac	tgc	agc	ttc	384
cag	atc	agc	acc	agc	gtg	cgc	aac	aag	atg	aag	cgc	gag	tac	gcc	ctg	432
ttc	tac	agc	ctg	gac	atc	gtg	ccc	att	gac	aac	gac	cag	acc	agc	tac	480
cgc	ctg	cgc	agc	tgc	aac	aca	tgc	atc	atc	acc	cag	gcc	tgc	ccc	aag	528
gtg	agc	ttc	gag	ccc	atc	ccc	atc	cac	ttc	tgc	gcc	ccc	gcc	ggc	ttc	576
gcc	att	ctg	aag	tgc	aac	aac	aag	acc	ttc	aat	ggc	acc	ggc	ccc	tgc	624
acc	aac	gtg	agc	acc	gtg	cag	tgc	acc	cac	gga	att	cgc	ccc	gtg	gtg	672
agc	acc	cag	ctg	ctg	ctg	aat	ggc	agc	ctg	gcc	gag	gag	gag	gtg	gtg	720
atc	aga	tct	gag	aat	ttc	acc	aat	acc	gcc	aag	acc	atc	atc	gtg	cag	768
ctg	aac	gag	agc	gtg	gag	atc	aat	tgc	acc	cgc	ccc	aat	aac	acc	acc	816
cgc	aag	agc	atc	cac	atc	ggc	cct	ggc	cgc	gcc	ttc	tac	acc	acc	ggc	864
gac	atc	atc	ggc	gac	atc	cgc	cag	gcc	cac	tgc	aat	atc	tct	aga	acc	912
aat	tgg	acc	aat	acc	ctg	aag	cgc	gtg	gcc	gag	aag	ctg	cgc	gag	aag	960
ttc	aac	aat	acc	acc	atc	gtg	ttc	aat	cag	agc	tcc	ggc	ggc	gac	ccc	1008
gag	atc	gtg	atg	cac	agc	ttc	aat	tgc	ggc	ggc	gag	ttc	ttc	tac	tgc	1056
aat	acc	acc	cag	ctg	ttc	aat	agc	acc	tgg	aat	gag	acc	aat	agc	gag	1104

ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag
 atc atc aac atg tgg cag gag gtg ggc aag gcc atg tac gcc ccc ccc
 atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg
 acc cgc gac ggc agc gac aac tcg ag

 <210> 50
 <211> 425
 <212> PRT
 <213> Artificial Sequence

 <400> 50
 Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1 5 10 15
 Val Trp Lys Asp Ala Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
 20 25 30
 Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
 35 40 45
 Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
 50 55 60
 Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
 65 70 75 80
 Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
 85 90 95
 Pro Leu Cys Val Thr Leu Gln Cys Thr Lys Leu Lys Gln Ser Thr Asp
 100 105 110
 Thr Gln Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
 115 120 125
 Gln Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
 130 135 140
 Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Gln Thr Ser Tyr
 145 150 155 160
 Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
 165 170 175
 Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
 180 185 190
 Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
 195 200 205
 Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
 210 215 220
 Ser Thr Gln Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
 225 230 235 240
 Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
 245 250 255
 Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
 260 265 270
 Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
 275 280 285
 Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
 290 295 300
 Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
 305 310 315 320
 Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
 325 330 335
 Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
 340 345 350
 Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
 355 360 365
 Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
 370 375 380

Ile	Ile	Asn	Met	Trp	Gln	Glu	Val	Gly	Lys	Ala	Met	Tyr	Ala	Pro	Pro
385					390				395					400	
Ile	Gly	Gly	Gln	Ile	Lys	Cys	Leu	Ser	Asn	Ile	Thr	Gly	Leu	Leu	
						405				410				415	
Thr	Arg	Asp	Gly	Gly	Ser	Asp	Asn	Ser							
						420			425						

<210> 51
<211> 144
<212> PRT
<213> Artificial Sequence

<400> 51

gct	agc	gcg	gcc	gac	cgc	ctg	tgg	gtg	acc	gtg	tac	tac	ggc	gtg	ccc	48
gtg	tgg	aag	gac	gcc	acc	acc	acc	ctg	ttc	tgc	gcc	agc	gac	gcc	aag	96
gcc	tac	gac	acc	gag	gtg	cac	aac	gtg	tgg	gcc	acc	cac	gca	tgc	gtg	144
ccc	acc	gac	ccc	aac	ccc	cag	gag	gtg	gtg	ctg	ggc	aac	gtg	acc	gag	192
aat	ttc	aac	atg	ggc	aag	aac	aat	atg	gtg	gag	cag	atg	cac	gag	aat	240
atc	288															
ccc	ctg	tgc	gtg	acc	ctg	aac	tgc	acc	aag	ccc	tgc	gtg	aag	ctg	acc	336
acc	aac	acc	cgc	tgg	ggc	acc	cag	gag	atg	aag	aac	tgc	agc	ttc		384
cag	atc	atc	acc	acc	acc	acc	acc	acc	atg	aag	cgc	gag	tac	gcc	ctg	432
ttc	tac	atc	480													
cgc	ctg	cgc	acc	aca	ata	tcg	atc	atc	acc	cag	acc	acc	acc	acc	acc	528
gtg	agc	tcc	gag	ccc	atc	ccc	atc	ttc	tgc	gcc	ccc	gcc	ggc	ttc		576
gcc	atc	ctg	aag	tgc	aac	aac	aag	acc	ttc	aat	ggc	acc	ggc	ccc	tgc	624
acc	aac	gtg	agg	acc	gtg	cag	tgc	acc	cac	gga	att	cgc	ccc	gtg	gtg	672
agc	acc	cag	ctg	ctg	aac	ggc	acc	ctg	gcc	gag	gag	gag	gtg	gtg	gtg	720
atc	aga	tct	gag	aac	ttc	acc	768									
ctg	aac	gag	agg	acc	gtg	gag	atc	acc	tgc	acc	cgc	ccc	acc	acc	acc	816
cgc	aag	agg	acc	864												
gac	atc	atc	ggc	gac	atc	cgc	cag	acc	tgc	acc	acc	acc	acc	acc	acc	912
acc	tgg	acc	960													
ttc	acc	1008														
gag	atc	gtg	atg	cac	acc	1056										
acc	acc	acc	cag	ctg	ttc	acc	1104									
ggc	acc	acc	act	agt	ggc	acc	1152									
atc	atc	acc	atg	tgg	cag	gag	gtg	ggc	aag	acc	atg	tac	gcc	ccc	ccc	1200
acc	ggc	ggc	cag	acc	tgc	acc	1248									
acc	cgc	gac	ggc	acc	1277											

<210> 52
<211> 425
<212> PRT
<213> Artificial Sequence

<400> 52

Ala	Ser	Ala	Ala	Asp	Arg	Leu	Trp	Val	Thr	Val	Tyr	Tyr	Gly	Val	Pro
1				5					10					15	
Val	Trp	Lys	Asp	Ala	Thr	Thr	Thr	Leu	Phe	Cys	Ala	Ser	Asp	Ala	Lys
					20			25				30			
Ala	Tyr	Asp	Thr	Glu	Val	His	Asn	Val	Trp	Ala	Thr	His	Ala	Cys	Val
				35			40				45				
Pro	Thr	Asp	Pro	Asn	Pro	Gln	Glu	Val	Val	Leu	Gly	Asn	Val	Thr	Glu
				50			55			60					
Asn	Phe	Asn	Met	Gly	Lys	Asn	Asn	Met	Val	Glu	Gln	Met	His	Glu	Asp
	65				70				75			80			
Ile	Ile	Ser	Leu	Trp	Asp	Gln	Ser	Leu	Lys	Pro	Cys	Val	Lys	Leu	Thr
				85				90			95				

Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
 100 105 110
 Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
 115 120 125
 Gln Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
 130 135 140
 Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Gln Thr Ser Tyr
 145 150 155 160
 Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
 165 170 175
 Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
 180 185 190
 Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
 195 200 205
 Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
 210 215 220
 Ser Thr Gln Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
 225 230 235 240
 Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
 245 250 255
 Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
 260 265 270
 Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
 275 280 285
 Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
 290 295 300
 Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
 305 310 315 320
 Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
 325 330 335
 Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
 340 345 350
 Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
 355 360 365
 Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
 370 375 380
 Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
 385 390 395 400
 Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
 405 410 415
 Thr Arg Asp Gly Gly Ser Asp Asn Ser
 420 425

<210> 53

<211> 432

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (1)...(432)

<400> 53

tct aga gcg cta cct cca gga cca gcg ctt cct ggg cat gtg ggg ctg
 Ser Arg Ala Leu Pro Pro Gly Pro Ala Leu Pro Gly His Val Gly Leu
 1 5 10 15

48

ctc cgg caa gct gat ctg cac cac ggc cgt gcc ctg gaa cgc cag ctg
 Leu Arg Gln Ala Asp Leu His His Gly Arg Ala Leu Glu Arg Gln Leu

96

20

25

30

gag caa caa gaa cct gag cca gat ttg gga caa cat gac ctg gat gga	144
Glu Gln Gln Glu Pro Glu Pro Asp Leu Gly Gln His Asp Leu Asp Gly	
35 40 45	
gtg gga gca gat cag caa cta cac cga gat cat cta cag cct gat	192
Val Gly Ala Arg Asp Gln Gln Leu His Arg Asp His Leu Gln Pro Asp	
50 55 60	
cga gga gag cca gaa cca gca gga gaa cga gct gga cct gct cca	240
Arg Gly Glu Pro Glu Pro Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro	
65 70 75 80	
gct gga caa gtg ggc aag ctt gtg gaa ctg gtt caa cat cac caa ctg	288
Ala Gly Gln Val Gly Lys Leu Val Glu Leu Val Gln His His Gln Leu	
85 90 95	
gct gtg gta cat caa gat ttt cat cat gat cgt ggg cg ^g cct gat cg ^g	336
Ala Val Val His Gln Asp Phe His Asp Arg Gly Arg Pro Asp Arg	
100 105 110	
cct gc ^g cat cg ^t gtt cac cg ^t gct gag cat cg ^t gaa cc ^g cg ^t gc ^g cca	384
Pro Ala His Arg Val His Arg Ala Glu His Arg Glu Pro Arg Ala Pro	
115 120 125	
ggg cta cag ccc cct gag ctt cca gac cc ^g cct gc ^c cg ^t gc ^g cc ^g	432
Gly Leu Gln Pro Pro Glu Leu Pro Asp Pro Pro Ala Arg Ala Pro Arg	
130 135 140	
<210> 54	
<211> 144	
<212> PRT	
<213> Artificial Sequence	
<400> 54	
Ser Arg Ala Leu Pro Pro Gly Pro Ala Leu Pro Gly His Val Gly Leu	
1 5 10 15	
Leu Arg Gln Ala Asp Leu His His Gly Arg Ala Leu Glu Arg Gln Leu	
20 25 30	
Glu Gln Glu Pro Glu Pro Asp Leu Gly Gln His Asp Leu Asp Gly	
35 40 45	
Val Gly Ala Arg Asp Gln Gln Leu His Arg Asp His Leu Gln Pro Asp	
50 55 60	
Arg Gly Glu Pro Glu Pro Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro	
65 70 75 80	
Ala Gly Gln Val Gly Lys Leu Val Glu Leu Val Gln His His Gln Leu	
85 90 95	
Ala Val Val His Gln Asp Phe His His Asp Arg Gly Arg Pro Asp Arg	
100 105 110	
Pro Ala His Arg Val His Arg Ala Glu His Arg Glu Pro Arg Ala Pro	
115 120 125	
Gly Met Gln Pro Pro Glu Leu Pro Asp Pro Pro Ala Arg Val Thr Asp	
130 135 140	
<210> 55	
<211> 434	
<212> DNA	

<213> Artificial Sequence

<220>

<221> CDS

<222> (1)...(434)

<400> 55

tct	aga	gca	cta	cct	cca	gga	cca	gca	ctt	cct	ggg	cat	gtg	ggg	ctg		48
Ser	Arg	Ala	Leu	Pro	Pro	Gly	Pro	Ala	Leu	Pro	Gly	His	Val	Gly	Leu		
1				5					10				15				

ctc	cg	caa	gct	gat	ctg	cac	cac	ggc	cgt	gcc	ctg	gaa	cgc	cag	ctg		96
Leu	Arg	Gln	Ala	Asp	Leu	His	His	Gly	Arg	Ala	Leu	Glu	Arg	Gln	Leu		
20					25						30						

gag	caa	caa	gaa	cct	gag	cca	gat	ttg	gga	caa	cat	gac	ctg	gat	gga		144
Glu	Gln	Gln	Glu	Pro	Glu	Pro	Asp	Leu	Gly	Gln	His	Asp	Leu	Asp	Gly		
35					40						45						

gtg	gga	gca	gag	cga	cat	cag	caa	cta	cac	cga	gat	cat	ctg	cct	gat		192
Val	Gly	Ala	Arg	Asp	Gln	Gln	Leu	His	Arg	Asp	His	Leu	Gln	Pro	Asp		
50					55					60							

cga	gga	gag	cca	gaa	cca	gca	gga	gaa	gaa	cga	gct	gga	cct	gct	cca		240
Arg	Gly	Glu	Pro	Glu	Pro	Ala	Gly	Glu	Glu	Arg	Ala	Gly	Pro	Ala	Pro		
65					70					75					80		

gct	gga	caa	gtg	ggc	aag	ctt	gtg	gaa	ctg	gtt	caa	cat	cac	caa	ctg		288
Ala	Gly	Gln	Val	Gly	Lys	Leu	Val	Glu	Leu	Val	Gln	His	His	Gln	Leu		
85					90						95						

gct	gtg	gta	cat	caa	gat	ttt	cat	cat	gat	cgt	ggg	cg	cct	gat	cg		336
Ala	Val	Val	His	Gln	Asp	Phe	His	His	Asp	Arg	Gly	Arg	Pro	Asp	Arg		
100						105					110						

cct	gca	cat	cgt	gtt	cac	cgt	gct	gag	cat	cgt	gaa	ccg	cgt	gca		384	
Pro	Ala	His	Arg	Val	His	Arg	Ala	Glu	His	Arg	Glu	Pro	Arg	Ala	Pro		
115						120					125						

ggg	atg	cag	ccc	cct	gag	ctt	cca	gac	ccg	cct	gcc	cgt	gtg	acg	gat		432
Gly	Met	Gln	Pro	Pro	Glu	Leu	Pro	Asp	Pro	Pro	Ala	Arg	Val	Thr	Asp		
130						135					140						

cc 434

<210> 56

<211> 144

<212> PRT

<213> Artificial Sequence

<400> 56

Ser	Arg	Ala	Leu	Pro	Pro	Gly	Pro	Ala	Leu	Pro	Gly	His	Val	Gly	Leu		
1				5					10			15					
Leu	Arg	Gln	Ala	Asp	Leu	His	His	Gly	Arg	Ala	Leu	Glu	Arg	Gln	Leu		
20					25				30								
Glu	Gln	Gln	Glu	Pro	Glu	Pro	Asp	Leu	Gly	Gln	His	Asp	Leu	Asp	Gly		
35					40				45								
Val	Gly	Ala	Arg	Asp	Gln	Gln	Leu	His	Arg	Asp	His	Leu	Gln	Pro	Asp		
50					55				60								

Arg Gly Glu Pro Glu Pro Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro
 65 70 75 80
 Ala Gly Gln Val Gly Lys Leu Val Glu Leu Val Gln His His Gln Leu
 85 90 95
 Ala Val Val His Gln Asp Phe His His Asp Arg Gly Arg Pro Asp Arg
 100 105 110
 Pro Ala His Arg Val His Arg Ala Glu His Arg Glu Pro Arg Ala Pro
 115 120 125
 Gly Met Gln Pro Pro Glu Leu Pro Asp Pro Pro Ala Arg Val Thr Asp
 130 135 140

<210> 57
 <211> 281
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(281)

<400> 57

tct	aga	gcg	cta	cct	cca	gga	cca	gcg	ctt	cct	ggg	cat	gtg	ggg	ctg		48
Ser	Arg	Ala	Leu	Pro	Pro	Gly	Pro	Ala	Leu	Pro	Gly	His	Val	Gly	Leu		
1															15		

ctc	cgg	caa	gct	gat	ctg	cac	cac	ggc	cgt	gcc	ctg	gaa	cgc	cag	ctg		96
Leu	Arg	Gln	Ala	Asp	Leu	His	His	Gly	Arg	Ala	Leu	Glu	Arg	Gln	Leu		
															30		

gag	caa	caa	gaa	cct	gag	cca	gat	ttg	gga	caa	cat	gac	ctg	gat	gga		144
Glu	Gln	Gln	Glu	Pro	Glu	Pro	Asp	Leu	Gly	Gln	His	Asp	Leu	Asp	Gly		
															45		

gtg	gga	gcg	cga	gat	cag	caa	cta	cac	cga	gat	cat	cta	cag	cct	gat		192
Val	Gly	Ala	Arg	Asp	Gln	Gln	Leu	His	Arg	Asp	His	Leu	Gln	Pro	Asp		
															60		

cga	gga	gag	cca	gaa	cca	gca	gga	gaa	gaa	cga	gct	gga	cct	gct	cca		240
Arg	Gly	Glu	Pro	Glu	Pro	Ala	Gly	Glu	Glu	Arg	Ala	Gly	Pro	Ala	Pro		
															80		

gct	gga	caa	gtg	ggc	aag	ctt	gtg	tga	ctg	att	gag	gat	cc			281
Ala	Gly	Gln	Val	Gly	Lys	Leu	Val	*	Leu	Ile	Glu	Asp				
															90	

<210> 58
 <211> 92
 <212> PRT
 <213> Artificial Sequence

<400> 58

Ser	Arg	Ala	Leu	Pro	Pro	Gly	Pro	Ala	Leu	Pro	Gly	His	Val	Gly	Leu		
1															15		
Leu	Arg	Gln	Ala	Asp	Leu	His	His	Gly	Arg	Ala	Leu	Glu	Arg	Gln	Leu		
															30		
Glu	Gln	Gln	Glu	Pro	Glu	Pro	Asp	Leu	Gly	Gln	His	Asp	Leu	Asp	Gly		
															45		
Val	Gly	Ala	Arg	Asp	Gln	Gln	Leu	His	Arg	Asp	His	Leu	Gln	Pro	Asp		

50	55	60													
Arg	Gly	Glu	Pro	Glu	Pro	Ala	Gly	Glu	Glu	Arg	Ala	Gly	Pro	Ala	Pro
65					70				75						80
Ala	Gly	Gln	Val	Gly	Lys	Leu	Val	Leu	Ile	Glu	Asp				
									85		90				

<210> 59
<211> 272
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1) . . . (272)

<400> 59

atc gat tgc gcg acc tgc tgc tga tcg tgg ccc gca tcg tgg agc tgc
Ile Asp Cys Ala Thr Cys Cys * Ser Trp Pro Ala Ser Trp Ser Cys
1 5 10 15

```
tgg gcc ggc gcg gct ggg aga tcc tga agt act ggt gga acc tgc tcc 96
Trp Ala Gly Ala Ala Gly Arg Ser * Ser Thr Gly Gly Thr Cys Ser
          20                  25                  30
```

```

agt act gga gcc agg agc tga aga act ctg cag tga gcc tgc tga acg      144
Ser Thr Gly Ala Arg Ser * Arg Thr Leu Gln * Ala Cys * Thr
35          40

```

```

cca ccg cca tcg ccg tgg ccg agg gca ccg acc gcg tga tcg agg tgg      192
Pro Pro Pro Ser Pro Trp Pro Arg Ala Pro Thr Ala * Ser Arg Trp
        45          50          55

```

tgc agc gca tct ggc gcg gca tcc tgc aca tcc cca ccc gaa ttc gcc 240
 Cys Ser Ala Ser Gly Ala Ala Ser Cys Thr Ser Pro Pro Glu Phe Ala
 60 65 70

agg gct tcg agc gcg ccc tgc tgt aag gat cc 272
 Arg Ala Ser Ser Ala Pro Cys Cys Lys Asp
 75 80

<210> 60
<211> 84
<212> PRT
<213> Artificial Sequence

<400> 60

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<210> 61
<211> 798
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(798)

<400> 61
ctc gag cag cgg caa gga gat ttt ccg ccc cgg cgg cga cat gcg 48
Leu Glu Gln Arg Gln Gly Asp Phe Prō-Prō Arg Arg Arg Arg His Ala
1 5 10 15

cga caa ctg gcg cag cga gct gta caa gta caa ggt ggt gaa gat cga 96
Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
20 25 30

gcc cct ggg cat cgc ccc cac caa ggc caa gcc ccc cgt ggt gca gcc 144
Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
35 40 45

cga gaa gcc cgc cgt ggg cat cgg cgc tat gtt cct cgg ctt cct ggg 192
Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
50 55 60

cgc tgc agg cag cac cat ggg cgc cgc cag cct gac cct gac cgt gca 240
Arg Cys Arg Gln His His Gly Arg Arg Gln Pro Asp Pro Asp Arg Ala
65 70 75 80

ggc ccg cca gct gct gag cgg cat cgt gca gca gaa caa cct gct 288
Gly Pro Pro Ala Ala Glu Arg His Arg Ala Ala Ala Glu Gln Pro Ala
85 90 95

gcc cgc cat cga ggc cca gca gca cct gct cca gct gac cgt gtg ggg 336
Ala Arg His Arg Gly Pro Ala Ala Pro Ala Pro Ala Asp Arg Val Gly
100 105 110

cat caa gca gct cca ggc cct cgt gct ggc tct aga gcc cta cct cca 384
His Gln Ala Ala Pro Gly Pro Arg Ala Gly Ser Arg Ala Leu Pro Pro
115 120 125

gga cca gcc ctt cct ggg cat gtg ggg ctc ccg caa gct gat ctg 432
Gly Pro Ala Leu Pro Gly His Val Gly Leu Leu Arg Gln Ala Asp Leu
130 135 140

cac cac ggc cgt gcc ctg gaa cgc cat gg ga caa caa gaa cct gg 480
His His Gly Arg Ala Leu Glu Arg Gln Leu Glu Gln Gln Glu Pro Glu
145 150 155 160

cca gat ttg gga caa cat gac ctg gat gga gtg gga gcc cga gat cag 528
Pro Asp Leu Gly Gln His Asp Leu Asp Gly Val Gly Ala Arg Asp Gln
165 170 175

caa cta cac cga gat cat cta cag cct gat cga gga gag cca gaa cca 576
Gln Leu His Arg Asp His Leu Gln Pro Asp Arg Gly Glu Pro Glu Pro
180 185 190

```

gca gga gaa gaa cga gct gga cct gct cca gct gga caa gtg ggc aag Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro Ala Gly Gln Val Gly Lys 195 200 205	624
ctt gtg gaa ctg gtt caa cat cac caa ctg gct gtg gta cat caa gat Leu Val Glu Leu Val Gln His His Gln Leu Ala Val Val His Gln Asp 210 215 220	672
ttt cat cat gat cgt ggg cg ^g cct gat cg ^g cct gc ^g cat cgt gtt cac Phe His His Asp Arg Gly Arg Pro Asp Arg Pro Ala His Arg Val His 225 230 235 240	720
cgt gct gag cat cgt gaa ccg cgt g c g ^g cta cag ccc cct gag Arg Ala Glu His Arg Glu Pro Arg Ala Pro Gly Leu Gln Pro Pro Glu 245 250 255	768
ctt cca gac ccg cct gcc cgt gcc ccg cg ^g Leu Pro Asp Pro Pro Ala Arg Ala Pro Arg 260 265	798
<210> 62	
<211> 266	
<212> PRT	
<213> Artificial Sequence	
<400> 62	
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Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala 35 40 45	
Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly 50 55 60	
Arg Cys Arg Gln His His Gly Arg Arg Gln Pro Asp Pro Asp Arg Ala 65 70 75 80	
Gly Pro Pro Ala Ala Glu Arg His Arg Ala Ala Ala Glu Gln Pro Ala 85 90 95	
Ala Arg His Arg Gly Pro Ala Ala Pro Ala Pro Ala Asp Arg Val Gly 100 105 110	
His Gln Ala Ala Pro Gly Pro Arg Ala Gly Ser Arg Ala Leu Pro Pro 115 120 125	
Gly Pro Ala Leu Pro Gly His Val Gly Leu Leu Arg Gln Ala Asp Leu 130 135 140	
His His Gly Arg Ala Leu Glu Arg Gln Leu Glu Gln Gln Glu Pro Glu 145 150 155 160	
Pro Asp Leu Gly Gln His Asp Leu Asp Gly Val Gly Ala Arg Asp Gln 165 170 175	
Gln Leu His Arg Asp His Leu Gln Pro Asp Arg Gly Glu Pro Glu Pro 180 185 190	
Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro Ala Gly Gln Val Gly Lys 195 200 205	
Leu Val Glu Leu Val Gln His His Gln Leu Ala Val Val His Gln Asp 210 215 220	
Phe His His Asp Arg Gly Arg Pro Asp Arg Pro Ala His Arg Val His 225 230 235 240	
Arg Ala Glu His Arg Glu Pro Arg Ala Pro Gly Leu Gln Pro Pro Glu 245 250 255	

Leu Pro Asp Pro Pro Ala Arg Ala Pro Arg					
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cga caa ctg gcg cag cga gct gta caa gta caa ggt ggt gaa gat cga					96
Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg					
20	25	30			
gcc cct ggg cat cgc ccc cac caa ggc caa gcg ccg cgt ggt gca gcg					144
Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala					
35	40	45			
cga gaa gcg cgc cgt ggg cat cgg cgc tat gtt cct cgg ctt cct ggg					192
Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly					
50	55	60			
cgc tgc agg cag cac cat ggg cgc cgc cag cct gac cct gac cgt gca					240
Arg Cys Arg Gln His His Gly Arg Arg Gln Pro Asp Pro Asp Arg Ala					
65	70	75	80		
ggc ccc cca gct gct gag cgg cat cgt gca gca gca gaa caa cct gct					288
Gly Pro Pro Ala Ala Glu Arg His Arg Ala Ala Ala Glu Gln Pro Ala					
85	90	95			
gcg ccc cat cga ggc cca gca gca cct gct cca gct gac cgt gtg ggg					336
Ala Arg His Arg Gly Pro Ala Ala Pro Ala Pro Ala Asp Arg Val Gly					
100	105	110			
cat caa gca gct cca ggc ccc cgt gct ggc tct aga gcg cta cct cca					384
His Gln Ala Ala Pro Gly Pro Arg Ala Gly Ser Arg Ala Leu Pro Pro					
115	120	125			
gga cca gcg ctt cct ggg cat gtg ggg ctg ctc cgg caa gct gat ctg					432
Gly Pro Ala Leu Pro Gly His Val Gly Leu Leu Arg Gln Ala Asp Leu					
130	135	140			
cac cac ggc cgt gcc ctg gaa cgc cag ctg gag caa caa gaa cct gag					480
His His Gly Arg Ala Leu Glu Arg Gln Leu Glu Gln Gln Glu Pro Glu					
145	150	155	160		
cca gat ttg gga caa cat gac ctg gat gga gtg gga gcg cga gat cag					528
Pro Asp Leu Gly Gln His Asp Leu Asp Gly Val Gly Ala Arg Asp Gln					
165	170	175			
caa cta cac cga gat cat cta cag cct gat cga gga gag cca gaa cca					576
Gln Leu His Arg Asp His Leu Gln Pro Asp Arg Gly Glu Pro Glu Pro					

180	185	190	
gca gga gaa gaa cga gct gga cct gct cca gct gga caa gtg ggc aag Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro Ala Gly Gln Val Gly Lys 195	200	205	624
ctt gtg gaa ctg gtt caa cat cac caa ctg gct gtg gta cat caa gat Leu Val Glu Leu Val Gln His His Gln Leu Ala Val Val His Gln Asp 210	215	220	672
ttt cat cat gat cgt ggg cgg cct gat cgg cct gcg cat cgt gtt cac Phe His His Asp Arg Gly Arg Pro Asp Arg Pro Ala His Arg Val His 225	230	235	720
cgt gct gag cat cgt gaa ccg cgt gcg cca ggg atg cag ccc cct gag Arg Ala Glu His Arg Glu Pro Arg Ala Pro Gly Met Gln Pro Pro Glu 245	250	255	768
ctt cca gac ccg cct gcc cgt gtg acg gat cc Leu Pro Asp Pro Pro Ala Arg Val Thr Asp 260	265		800
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Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala 35 40 45			
Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly 50 55 60			
Arg Cys Arg Gln His His Gly Arg Arg Gln Pro Asp Pro Asp Arg Ala 65 70 75 80			
Gly Pro Pro Ala Ala Glu Arg His Arg Ala Ala Ala Glu Gln Pro Ala 85 90 95			
Ala Arg His Arg Gly Pro Ala Ala Pro Ala Pro Ala Asp Arg Val Gly 100 105 110			
His Gln Ala Ala Pro Gly Pro Arg Ala Gly Ser Arg Ala Leu Pro Pro 115 120 125			
Gly Pro Ala Leu Pro Gly His Val Gly Leu Leu Arg Gln Ala Asp Leu 130 135 140			
His His Gly Arg Ala Leu Glu Arg Gln Leu Glu Gln Gln Glu Pro Glu 145 150 155 160			
Pro Asp Leu Gly Gln His Asp Leu Asp Gly Val Gly Ala Arg Asp Gln 165 170 175			
Gln Leu His Arg Asp His Leu Gln Pro Asp Arg Gly Glu Pro Glu Pro 180 185 190			
Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro Ala Gly Gln Val Gly Lys 195 200 205			
Leu Val Glu Leu Val Gln His His Gln Leu Ala Val Val His Gln Asp 210 215 220			
Phe His His Asp Arg Gly Arg Pro Asp Arg Pro Ala His Arg Val His 225 230 235			
			240

Arg Ala Glu His Arg Glu Pro Arg Ala Pro Gly Met Gln Pro Pro Glu
 245 250 255
 Leu Pro Asp Pro Pro Ala Arg Val Thr Asp
 260 265

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cga caa ctg gcg cag cga gct gta caa gta caa ggt ggt gaa gat cga 96
 Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
 20 25 30

gcc cct ggg cat cgc ccc cac caa ggc caa gcg ccg cgt ggt gca gcg 144
 Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
 35 40 45

cga gaa gcg cgc cgt ggg cat ccg cgc tat gtt cct ccg ctt cct ggg 192
 Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
 50 55 60

cgc tgc agg cag cac cat ggg cgc cgc cag cct gac cct gac cgt gca 240
 Arg Cys Arg Gln His His Gly Arg Arg Gln Pro Asp Pro Asp Arg Ala
 65 70 75 80

ggc ccg cca gct gct gag cgcat cgt gca gca gca gaa caa cct gct 288
 Gly Pro Pro Ala Ala Glu Arg His Arg Ala Ala Glu Gln Pro Ala
 85 90 95

gcg cgc cat cga ggc cca gca gca cct gct cca gct gac cgt gtg ggg 336
 Ala Arg His Arg Gly Pro Ala Ala Pro Ala Pro Ala Asp Arg Val Gly
 100 105 110

cat caa gca gct cca ggc ccg cgt gct ggc tct aga gcg cta cct cca 384
 His Gln Ala Ala Pro Gly Pro Arg Ala Gly Ser Arg Ala Leu Pro Pro
 115 120 125

gga cca gcg ctt cct ggg cat gtg ggg ctg ctc ccg caa gct gat ctg 432
 Gly Pro Ala Leu Pro Gly His Val Gly Leu Leu Arg Gln Ala Asp Leu
 130 135 140

cac cac ggc cgt gcc ctg gaa cgc cag ctg gag caa caa gaa cct gag 480
 His His Gly Arg Ala Leu Glu Arg Gln Leu Glu Gln Glu Pro Glu
 145 150 155 160

cca gat ttg gga caa cat gac ctg gat gga gtg gga gcg cga gat cag 528
 Pro Asp Leu Gly Gln His Asp Leu Asp Gly Val Gly Ala Arg Asp Gln
 165 170 175

caa cta cac cga gat cat cta cag cct gat cga gga gag cca gaa cca Gln Leu His Arg Asp His Leu Gln Pro Asp Arg Gly Glu Pro Glu Pro 180 185 190	576
gca gga gaa gaa cga gct gga cct gct cca gct gga caa gtg ggc aag Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro Ala Gly Gln Val Gly Lys 195 200 205	624
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Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala 35 40 45	
Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly 50 55 60	
Arg Cys Arg Gln His His Gly Arg Arg Gln Pro Asp Pro Asp Arg Ala 65 70 75 80	
Gly Pro Pro Ala Ala Glu Arg His Arg Ala Ala Ala Glu Gln Pro Ala 85 90 95	
Ala Arg His Arg Gly Pro Ala Ala Pro Ala Pro Ala Asp Arg Val Gly 100 105 110	
His Gln Ala Ala Pro Gly Pro Arg Ala Gly Ser Arg Ala Leu Pro Pro 115 120 125	
Gly Pro Ala Leu Pro Gly His Val Gly Leu Leu Arg Gln Ala Asp Leu 130 135 140	
His His Gly Arg Ala Leu Glu Arg Gln Leu Glu Gln Gln Glu Pro Glu 145 150 155 160	
Pro Asp Leu Gly Gln His Asp Leu Asp Gly Val Gly Ala Arg Asp Gln 165 170 175	
Gln Leu His Arg Asp His Leu Gln Pro Asp Arg Gly Glu Pro Glu Pro 180 185 190	
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gtg tgg aag gac gcc acc acc ctg ttc tgc gcc agc gac gcc aag			96	
Val Trp Lys Asp Ala Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys	20	25	30	
gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg			144	
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val	35	40	45	
ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag			192	
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu	50	55	60	
aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat			240	
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp	65	70	75	80
atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg acc			288	
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr	85	90	95	
ccc ctg tgc gtg acc ctg aac tgc acc aag ctg aag aac agc acc gac			336	
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp	100	105	110	
acc aac aac acc cgc tgg ggc acc cag gag atg aag aac tgc agc ttc			384	
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe	115	120	125	
aac atc agc acc agc gtg cgc aac aag atg aag cgc gag tac gcc ctg			432	
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu	130	135	140	
ttc tac agc ctg gac atc gtg ccc atc gac aac gac aac acc agc tac			480	
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr	145	150	155	160
cgc ctg cgc agc tgc aac aca tcg atc atc acc cag gcc tgc ccc aag			528	
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys	165	170	175	
gtg agc ttc gag ccc atc ccc atc cac ttc tgc gcc ccc gcc ggc ttc			576	
Val Ser Phe Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe	180	185	190	
gcc atc ctg aag tgc aac aac aag acc ttc aac ggc acc ggc ccc tgc			624	
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys	195	200	205	
acc aac gtg agc acc gtg cag tgc acc cac gga att cgc ccc gtg gtg			672	
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val	210	215	220	
agc acc cag ctg ctg aac ggc agc ctg gcc gag gag gag gtg gtg			720	
Ser Thr Gln Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val	225	230	235	240

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ctg aac gag agc gtg gag atc aac tgc acc cgc ccc aac aac aac acc Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr 260 265 270	816
cgc aag agc atc cac atc ggc cct ggc cgc gcc ttc tac acc acc ggc Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly 275 280 285	864
gac atc atc ggc gac atc cgc cag gcc cac tgc aac atc tct aga acc Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr 290 295 300	912
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ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc ggc gac ccc Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro 325 330 335	1008
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gcc aag cgc cgc gtg gtg cag cgc gag aag cgc gcc gtg ggc atc ggc Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly Ile Gly 465 470 475 480	1440

gct atg ttc ctc ggc ttc ctg ggc gct gca ggc agc acc atg ggc gcc Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala 485 490 495	1488
gcc agc ctg acc ctg acc gtg cag gcc cgc cag ctg ctg agc ggc atc Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile 500 505 510	1536
gtg cag cag cag aac aac ctg ctg cgc gcc atc gag gcc cag cag cac Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His 515 520 525	1584
ctg ctc cag ctg acc gtg tgg ggc atc aag cag ctc cag gcc cgc gtg Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val 530 535 540	1632
ctg gct cta gag cgc tac ctc cag gac cag cgc ttc ctg ggc atg tgg Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg Phe Leu Gly Met Trp 545 550 555 560	1680
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agc tgg agc aac aag aac ctg agc cag att tgg gac aac atg acc tgg Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp Asp Asn Met Thr Trp 580 585 590	1776
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ctg atc gag gag agc cag aac cag cag gag aag aac gag ctg gac ctg Leu Ile Glu Glu Ser Gln Asn Gln Glu Lys Asn Glu Leu Asp Leu 610 615 620	1872
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Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val 35 40 45	
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu 50 55 60	
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp 65 70 75 80	
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr	

85	90	95
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp		
100	105	110
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe		
115	120	125
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu		
130	135	140
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr		
145	150	155
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys		
165	170	175
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe		
180	185	190
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys		
195	200	205
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val		
210	215	220
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val		
225	230	235
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln		
245	250	255
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr		
260	265	270
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly		
275	280	285
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr		
290	295	300
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys		
305	310	315
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro		
325	330	335
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys		
340	345	350
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu		
355	360	365
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln		
370	375	380
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro		
385	390	395
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu		
405	410	415
Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Ser Gly Lys Glu Ile Phe		
420	425	430
Arg Pro Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr		
435	440	445
Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys		
450	455	460
Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly Ile Gly		
465	470	475
Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala		
485	490	495
Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile		
500	505	510
Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His		
515	520	525
Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val		
530	535	540
Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg Phe Leu Gly Met Trp		
545	550	555
		560

Gly	Cys	Ser	Gly	Lys	Leu	Ile	Cys	Thr	Thr	Ala	Val	Pro	Trp	Asn	Ala
				565					570					575	
Ser	Trp	Ser	Asn	Lys	Asn	Leu	Ser	Gln	Ile	Trp	Asp	Asn	Met	Thr	Trp
				580					585				590		
Met	Glu	Trp	Glu	Arg	Glu	Ile	Ser	Asn	Tyr	Thr	Glu	Ile	Ile	Tyr	Ser
				595				600				605			
Leu	Ile	Glu	Glu	Ser	Gln	Asn	Gln	Gln	Glu	Lys	Asn	Glu	Leu	Asp	Leu
				610				615			620				
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 Val Trp Lys Asp Ala Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
 20 25 30

gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg 144
 Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
 35 40 45

ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag 192
 Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
 50 55 60

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aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat 240
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
   65           70           75           80

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85 90 95

ccc ctg tgc gtg acc ctg aac tgc acc aag ctg aag aac agc acc gac 336
 Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
 100 105 110

acc aac aac acc cgc tgg ggc acc cag gag atg aag aac tgc agc ttc
 Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
 115 120 125

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Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
   130          135          140

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170				175
gtg agc ttc gag ccc atc ccc atc cac ttc tgc gcc ccc gcc ggc ttc Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe 180				576
185				190
gcc atc ctg aag tgc aac aac aag acc ttc aac ggc acc ggc ccc tgc Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys 195				624
200				205
acc aac gtg agc acc gtg cag tgc acc cac gga att cgc ccc gtg gtg Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val 210				672
215				220
agc acc cag ctg ctg aac ggc agc ctg gcc gag gag gag gtg gtg Ser Thr Gln Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val 225				720
230				235
atc aga tct gag aac ttc acc aac aac gcc aag acc atc atc gtg cag Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln 245				768
250				255
ctg aac gag agc gtg gag atc aac tgc acc cgc ccc aac aac aac acc Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr 260				816
265				270
cgc aag agc atc cac atc ggc cct ggc cgc gcc ttc tac acc acc ggc Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Gly 275				864
280				285
gac atc atc ggc gac atc cgc cag gcc cac tgc aac atc tct aga acc Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr 290				912
295				300
aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg cgc gag aag Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys 305				960
310				315
320				
ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc ggc gac ccc Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro 325				1008
330				335
gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc ttc tac tgc Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys 340				1056
345				350
aac acc acc cag ctg ttc aac agc acc tgg aac gag acc aac agc gag Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu 355				1104
360				365
ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln 370				1152
375				380
atc atc aac atg tgg cag gag gtg ggc aag gcc atg tac gcc ccc ccc				1200

Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro			
385	390	395	400
atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg			1248
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu			
405	410	415	
acc cgc gac ggc ggc agc gac aac tcg agc agc ggc aag gag att ttc			1296
Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Ser Gly Lys Glu Ile Phe			
420	425	430	
cgc ccc ggc ggc gac atg cgc gac aac tgg cgc agc gag ctg tac			1344
Arg Pro Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr			
435	440	445	
aag tac aag gtg gtg aag atc gag ccc ctg ggc atc gcc ccc acc aag			1392
Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys			
450	455	460	
gcc aag cgc cgc gtg gtg cag cgc gag aag cgc gcc gtg ggc atc ggc			1440
Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly Ile Gly			
465	470	475	480
gct atg ttc ctc ggc ttc ctg ggc gct gca ggc agc acc atg ggc gcc			1488
Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala			
485	490	495	
gcc agc ctg acc ctg acc gtg cag gcc cgc cag ctg ctg agc ggc atc			1536
Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile			
500	505	510	
gtg cag cag cag aac aac ctg ctg cgc gcc atc gag gcc cag cag cac			1584
Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His			
515	520	525	
ctg ctc cag ctg acc gtg tgg ggc atc aag cag ctc cag gcc cgc gtg			1632
Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val			
530	535	540	
ctg gct cta gag cgc tac ctc cag gac cag cgc ttc ctg ggc atg tgg			1680
Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg Phe Leu Gly Met Trp			
545	550	555	560
ggc tgc tcc ggc aag ctg atc tgc acc acg gcc gtg ccc tgg aac gcc			1728
Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala			
565	570	575	
agc tgg agc aac aag aac ctg agc cag att tgg gac aac atg acc tgg			1776
Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp Asp Asn Met Thr Trp			
580	585	590	
atg gag tgg gag cgc gag atc agc aac tac acc gag atc atc tac agc			1824
Met Glu Trp Glu Arg Glu Ile Ser Asn Tyr Thr Glu Ile Ile Tyr Ser			
595	600	605	
ctg atc gag gag agc cag aac cag cag gag aag aac gag ctg gac ctg			1872
Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Leu Asp Leu			
610	615	620	

ctc cag ctg gac aag tgg gca agc ttg tgg aac tgg ttc aac atc acc Leu Gln Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe Asn Ile Thr 625 630 635 640	1920
aac tgg ctg tgg tac atc aag att ttc atc atg atc gtg ggc ggc ctg Asn Trp Leu Trp Tyr Ile Lys Ile Phe Ile Met Ile Val Gly Gly Leu 645 650 655	1968
atc ggc ctg cgc atc gtg ttc acc gtg ctg agc atc gtg aac cgc gtg Ile Gly Leu Arg Ile Val Phe Thr Val Leu Ser Ile Val Asn Arg Val 660 665 670	2016
cgc cag gga tgc agc ccc ctg agc ttc cag acc cgc ctg ccc gtg tga Arg Gln Gly Cys Ser Pro Leu Ser Phe Gln Thr Arg Leu Pro Val *	2064
675 680 685	
cgg atc c Arg Ile	2071

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35 40 45	
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu	
50 55 60	
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp	
65 70 75 80	
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr	
85 90 95	
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp	
100 105 110	
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe	
115 120 125	
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu	
130 135 140	
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr	
145 150 155 160	
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys	
165 170 175	
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe	
180 185 190	
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys	
195 200 205	
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val	
210 215 220	
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val	
225 230 235 240	
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln	
245 250 255	

Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
 260 265 270
 Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
 275 280 285
 Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
 290 295 300
 Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
 305 310 315 320
 Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
 325 330 335
 Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
 340 345 350
 Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
 355 360 365
 Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
 370 375 380
 Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
 385 390 395 400
 Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu
 405 410 415
 Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Ser Gly Lys Glu Ile Phe
 420 425 430
 Arg Pro Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr
 435 440 445
 Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys
 450 455 460
 Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly Ile Gly
 465 470 475 480
 Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala
 485 490 495
 Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile
 500 505 510
 Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His
 515 520 525
 Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val
 530 535 540
 Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg Phe Leu Gly Met Trp
 545 550 555 560
 Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala
 565 570 575
 Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp Asp Asn Met Thr Trp
 580 585 590
 Met Glu Trp Glu Arg Glu Ile Ser Asn Tyr Thr Glu Ile Ile Tyr Ser
 595 600 605
 Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Leu Asp Leu
 610 615 620
 Leu Gln Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe Asn Ile Thr
 625 630 635 640
 Asn Trp Leu Trp Tyr Ile Lys Ile Phe Ile Met Ile Val Gly Gly Leu
 645 650 655
 Ile Gly Leu Arg Ile Val Phe Thr Val Leu Ser Ile Val Asn Arg Val
 660 665 670
 Arg Gln Gly Cys Ser Pro Leu Ser Phe Gln Thr Arg Leu Pro Val Arg
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 Ile

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 <213> Artificial Sequence

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acc aac gtg agc acc gtg cag tgc acc cac gga att cgc ccc gtg gtg	672
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val	
210 215 220	
 agc acc cag ctg ctg aac ggc agc ctg gcc gag gag gag gtg gtg	720
Ser Thr Gln Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val	
225 230 235 240	
 atc aga tct gag aac ttc acc aac aac gcc aag acc atc atc gtg cag	768
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln	
245 250 255	
 ctg aac gag agc gtg gag atc aac tgc acc cgc ccc aac aac aac acc	816
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr	
260 265 270	
 cgc aag agc atc cac atc ggc cct ggc cgc gcc ttc tac acc acc ggc	864
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly	
275 280 285	
 gac atc atc ggc gac atc cgc cag gcc cac tgc aac atc tct aga acc	912
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr	
290 295 300	
 aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg cgc gag aag	960
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys	
305 310 315 320	
 ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc ggc gac ccc	1008
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro	
325 330 335	
 gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc ttc tac tgc	1056
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys	
340 345 350	
 aac acc acc cag ctg ttc aac agc acc tgg aac gag acc aac agc gag	1104
Asn Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu	
355 360 365	
 ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag	1152
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln	
370 375 380	
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Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro	
385 390 395 400	
 atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg	1248
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu	
405 410 415	
 acc cgc gac ggc ggc agc gac aac tcg agc agc ggc aag gag att ttc	1296
Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Ser Gly Lys Glu Ile Phe	
420 425 430	
 cgc ccc ggc ggc gac atg cgc gac aac tgg cgc agc gag ctg tac	1344
Arg Pro Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr	

435	440	445	
aag tac aag gtg gtg aag atc gag ccc ctg ggc atc gcc ccc acc aag Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys 450	455	460	1392
gcc aag cgc cgc gtg gtg cag cgc gag aag cgc gcc gtc ggc atc ggc Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly Ile Gly 465	470	475	1440
gct atg ttc ctc ggc ttc ctg ggc gct gca ggc agc acc atg ggc gcc Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala 485	490	495	1488
gcc agc ctg acc ctg acc gtg cag gcc cgc cag ctg ctg agc ggc atc Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile 500	505	510	1536
gtg cag cag cag aac aac ctg ctg cgc gcc atc gag gcc cag cag cac Val Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His 515	520	525	1584
ctg ctc cag ctg acc gtg tgg ggc atc aag cag ctc cag gcc cgc gtc Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val 530	535	540	1632
ctg gct cta gag cgc tac ctc cag gac cag cgc ttc ctg ggc atg tgg Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg Phe Leu Gly Met Trp 545	550	555	1680
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agc tgg agc aac aag aac ctg agc cag att tgg gac aac atg acc tgg Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp Asp Asn Met Thr Trp 580	585	590	1776
atg gag tgg gag cgc gag atc agc aac tac acc gag atc atc tac agc Met Glu Trp Glu Arg Glu Ile Ser Asn Tyr Thr Glu Ile Ile Tyr Ser 595	600	605	1824
ctg atc gag gag agc cag aac cag cag gag aag aac gag ctg gac ctg Leu Ile Glu Glu Ser Gln Asn Gln Glu Lys Asn Glu Leu Asp Leu 610	615	620	1872
ctc cag ctg gac aag tgg gca agc ttg tgg aac tgg ttc aac atc acc Leu Gln Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe Asn Ile Thr 625	630	635	1920
aac tgg ctg tgg tac atc aag att ttc atc atg atc gtc ggc ggc ctg Asn Trp Leu Trp Tyr Ile Lys Ile Phe Ile Met Ile Val Gly Gly Leu 645	650	655	1968
atc ggc ctg cgc atc gtg ttc acc gtg ctg agc atc gtg aac cgc gtg Ile Gly Leu Arg Ile Val Phe Thr Val Leu Ser Ile Val Asn Arg Val 660	665	670	2016
cgc cag ggc tac agc ccc ctg agc ttc cag acc cgc ctg ccc gtg ccc			2064

Arg Gln Gly Tyr Ser Pro Leu Ser Phe Gln Thr Arg Leu Pro Val Pro	675	680	685	
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Arg Gly Pro Asp Arg Pro Glu Gly Ile Glu Glu Gly Gly Glu Arg				
690	695	700		
gac cgc gac cgc agc acc cgc ctg gtg acc ggc ttc ctg ccc ctg atc				2160
Asp Arg Asp Arg Ser Thr Arg Leu Val Thr Gly Phe Leu Pro Leu Ile				
705	710	715	720	
tgg gac gac ctg cgc agc ctg ttc ctg ttc agc tac cat cga ttg cgc				2208
Trp Asp Asp Leu Arg Ser Leu Phe Leu-Phe Ser Tyr His Arg Leu Arg				
725	730	735		
gac ctg ctg ctg atc gtg gcc cgc atc gtg gag ctg ctg ggc cgg cgc				2256
Asp Leu Leu Leu Ile Val Ala Arg Ile Val Glu Leu Leu Gly Arg Arg				
740	745	750		
ggc tgg gag atc ctg aag tac tgg tgg aac ctg ctc cag tac tgg agc				2304
Gly Trp Glu Ile Leu Lys Tyr Trp Trp Asn Leu Leu Gln Tyr Trp Ser				
755	760	765		
cag gag ctg aag aac tct gca gtg agc ctg ctg aac gcc acc gcc atc				2352
Gln Glu Leu Lys Asn Ser Ala Val Ser Leu Leu Asn Ala Thr Ala Ile				
770	775	780		
gcc gtg gcc gag ggc acc gac cgc gtg atc gag gtg gtg cag cgc atc				2400
Ala Val Ala Glu Gly Thr Asp Arg Val Ile Glu Val Val Gln Arg Ile				
785	790	795	800	
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Trp Arg Gly Ile Leu His Ile Pro Thr Arg Ile Arg Gln Gly Phe Glu				
805	810	815		
cgc gcc ctg ctg taa gga tcc				2469
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820				
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35 40 45				
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu				
50 55 60				
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp				
65 70 75 80				
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr				
85 90 95				
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp				

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Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe			
115	120	125	
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu			
130	135	140	
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr			
145	150	155	160
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys			
165	170	175	
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe			
180	185	190	
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys			
195	200	205	
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val			
210	215	220	
Ser Thr Gln Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val			
225	230	235	240
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln			
245	250	255	
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr			
260	265	270	
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly			
275	280	285	
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr			
290	295	300	
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys			
305	310	315	320
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro			
325	330	335	
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys			
340	345	350	
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu			
355	360	365	
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln			
370	375	380	
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro			
385	390	395	400
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu			
405	410	415	
Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Ser Gly Lys Glu Ile Phe			
420	425	430	
Arg Pro Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr			
435	440	445	
Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys			
450	455	460	
Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly Ile Gly			
465	470	475	480
Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala			
485	490	495	
Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile			
500	505	510	
Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His			
515	520	525	
Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val			
530	535	540	
Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg Phe Leu Gly Met Trp			
545	550	555	560
Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala			
565	570	575	

Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp Asp Asn Met Thr Trp
 580 585 590
 Met Glu Trp Glu Arg Glu Ile Ser Asn Tyr Thr Glu Ile Ile Tyr Ser
 595 600 605
 Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Leu Asp Leu
 610 615 620
 Leu Gln Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe Asn Ile Thr
 625 630 635 640
 Asn Trp Leu Trp Tyr Ile Lys Ile Phe Ile Met Ile Val Gly Gly Leu
 645 650 655
 Ile Gly Leu Arg Ile Val Phe Thr Val Leu Ser Ile Val Asn Arg Val
 660 665 670
 Arg Gln Gly Tyr Ser Pro Leu Ser Phe Gln Thr Arg Leu Pro Val Pro
 675 680 685
 Arg Gly Pro Asp Arg Pro Glu Gly Ile Glu Glu Glu Gly Gly Glu Arg
 690 695 700
 Asp Arg Asp Arg Ser Thr Arg Leu Val Thr Gly Phe Leu Pro Leu Ile
 705 710 715 720
 Trp Asp Asp Leu Arg Ser Leu Phe Leu Phe Ser Tyr His Arg Leu Arg
 725 730 735
 Asp Leu Leu Leu Ile Val Ala Arg Ile Val Glu Leu Leu Gly Arg Arg
 740 745 750
 Gly Trp Glu Ile Leu Lys Tyr Trp Trp Asn Leu Leu Gln Tyr Trp Ser
 755 760 765
 Gln Glu Leu Lys Asn Ser Ala Val Ser Leu Leu Asn Ala Thr Ala Ile
 770 775 780
 Ala Val Ala Glu Gly Thr Asp Arg Val Ile Glu Val Val Gln Arg Ile
 785 790 795 800
 Trp Arg Gly Ile Leu His Ile Pro Thr Arg Ile Arg Gln Gly Phe Glu
 805 810 815
 Arg Ala Leu Leu Gly Ser
 820

<210> 73

<211> 1431

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (1)...(1431)

<400> 73

gct	agc	gcg	gcc	gac	cgc	ctg	tgg	gtg	acc	gtg	tac	tac	ggc	gtg	ccc	
Ala	Ser	Ala	Ala	Asp	Arg	Leu	Trp	Val	Thr	Val	Tyr	Tyr	Gly	Val	Pro	
1						5				10			15			

48

gtg	tgg	aag	gac	gcc	acc	acc	acc	ctg	ttc	tgc	gcc	agc	gac	gcc	aag	
Val	Trp	Lys	Asp	Ala	Thr	Thr	Leu	Phe	Cys	Ala	Ser	Asp	Ala	Lys		
20							25				30					

96

gcc	tac	gac	acc	gag	gtg	cac	aac	gtg	tgg	gcc	acc	cac	gcg	tgc	gtg	
Ala	Tyr	Asp	Thr	Glu	Val	His	Asn	Val	Trp	Ala	Thr	His	Ala	Cys	Val	
35							40				45					

144

ccc	acc	gac	ccc	aac	ccc	cag	gag	gtg	gtg	ctg	ggc	aac	gtg	acc	gag	
Pro	Thr	Asp	Pro	Asn	Pro	Gln	Glu	Val	Val	Leu	Gly	Asn	Val	Thr	Glu	
50							55				60					

192

aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp 65 70 75 80	240
atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg acc Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr 85 90 95	288
ccc ctg tgc gtg acc ctg aac tgc acc aag ctg aag aac agc acc gac Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp 100 105 110	336
acc aac aac acc cgc tgg ggc acc cag-gag atg aag aac tgc agc ttc Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe 115 120 125	384
aac atc agc acc agc gtg cgc aac aag atg aag cgc gag tac gcc ctg Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu 130 135 140	432
ttc tac agc ctg gac atc gtg ccc atc gac aac gac aac acc agc tac Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr 145 150 155 160	480
cgc ctg cgc agc tgc aac aca tcg atc atc acc cag gcc tgc ccc aag Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys 165 170 175	528
gtg agc ttc gag ccc atc ccc atc cac ttc tgc gcc ccc gcc ggc ttc Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe 180 185 190	576
gcc atc ctg aag tgc aac aac aag acc ttc aac ggc acc ggc ccc tgc Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys 195 200 205	624
acc aac gtg agc acc gtg cag tgc acc cac gga att cgc ccc gtg gtg Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val 210 215 220	672
agc acc cag ctg ctg aac ggc agc ctg gcc gag gag gag gtg gtg Ser Thr Gln Leu Leu Asn Gly Ser Leu Ala Glu Glu Val Val 225 230 235 240	720
atc aga tct gag aac ttc acc aac aac gcc aag acc atc atc gtg cag Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln 245 250 255	768
ctg aac gag agc gtg gag atc aac tgc acc cgc ccc aac aac aac acc Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr 260 265 270	816
cgc aag agc atc cac atc ggc cct ggc cgc gcc ttc tac acc acc ggc Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly 275 280 285	864
gac atc atc ggc gac atc cgc cag gcc cac tgc aac atc tct aga acc Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr 290 295 300	912

aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg cgc gag aag Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys 305 310 315 320	960
ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc ggc gac ccc Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro 325 330 335	1008
gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc ttc tac tgc Glu Ile Val Met His Ser Phe Asn Cys Gly Glu Phe Phe Tyr Cys 340 345 350	1056
aac acc acc cag ctg ttc aac agc acc tgg aac gag acc aac agc gag Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu 355 360 365	1104
ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln 370 375 380	1152
atc atc aac atg tgg cag gag gtg ggc aag gcc atg tac gcc ccc ccc Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro 385 390 395 400	1200
atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu 405 410 415	1248
acc cgc gac ggc ggc agc gac aac tcg agc agc ggc aag gag att ttc Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Gly Lys Glu Ile Phe 420 425 430	1296
cgc ccc ggc ggc gac atg cgc gac aac tgg cgc agc gag ctg tac Arg Pro Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr 435 440 445	1344
aag tac aag gtg gtg aag atc gag ccc ctg ggc atc gcc ccc acc aag Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys 450 455 460	1392
gcc aag cgc cgc gtg gtg cag cgc gag aag cgc gcc tag Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala *	1431
465 470 475	
<210> 74	
<211> 476	
<212> PRT	
<213> Artificial Sequence	
<400> 74	
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro	
1 5 10 15	
Val Trp Lys Asp Ala Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys	
20 25 30	
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val	
35 40 45	
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu	

50	55	60
Asn Phe Asn Met Gly Lys Asn Asn Met Val	Glu Gln Met His Glu Asp	
65	70	75
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr		80
85	90	95
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp		
100	105	110
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe		
115	120	125
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu		
130	135	140
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asn Thr Ser Tyr		
145	150	155
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys		160
165	170	175
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe		
180	185	190
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys		
195	200	205
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val		
210	215	220
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val		
225	230	235
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln		240
245	250	255
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Thr		
260	265	270
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly		
275	280	285
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr		
290	295	300
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys		
305	310	315
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro		320
325	330	335
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys		
340	345	350
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu		
355	360	365
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln		
370	375	380
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro		
385	390	395
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu		400
405	410	415
Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Ser Gly Lys Glu Ile Phe		
420	425	430
Arg Pro Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr		
435	440	445
Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys		
450	455	460
Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala		
465	470	475

<210> 75

<211> 1038

<212> DNA

<213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)...(1038)

<400> 75

gcc gtg ggc atc ggc gct atg ttc ctc ggc ttc ctg ggc gct gca ggc	48
Ala Val Gly Ile Gly Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly	
1 5 10 15	

agc acc atg ggc gcc gcc agc ctg acc ctg acc gtg cag gcc cgcc cag

Ser Thr Met Gly Ala Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln	96
20 25 30	

ctg ctg agc ggc atc gtg cag cag aac aac ctg ctg cgcc gcc atc

Leu Leu Ser Gly Ile Val Gln Gln Asn Asn Leu Leu Arg Ala Ile	144
35 40 45	

gag gcc cag cag cac ctg ctc cag ctg acc gtg tgg ggc atc aag cag

Glu Ala Gln Gln His Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln	192
50 55 60	

ctc cag gcc cgcc gtg ctg gct cta gag cgcc tac ctc cag gac cag cgcc

Leu Gln Ala Arg Val Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg	240
65 70 75 80	

ttc ctg ggc atg tgg ggc tgc tcc ggc aag ctg atc tgc acc acg gcc

Phe Leu Gly Met Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala	288
85 90 95	

gtg ccc tgg aac gcc agc tgg agc aac aag aac ctg agc cag att tgg

Val Pro Trp Asn Ala Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp	336
100 105 110	

gac aac atg acc tgg atg gag tgg gag cgcc gag atc agc aac tac acc

Asp Asn Met Thr Trp Met Glu Trp Glu Arg Glu Ile Ser Asn Tyr Thr	384
115 120 125	

gag atc atc tac agc ctg atc gag gag agc cag aac cag cag gag aag

Glu Ile Ile Tyr Ser Leu Ile Glu Glu Ser Gln Asn Gln Glu Lys	432
130 135 140	

aac gag ctg gac ctg ctc cag ctg gac aag tgg gca agc ttg tgg aac

Asn Glu Leu Asp Leu Leu Gln Leu Asp Lys Trp Ala Ser Leu Trp Asn	480
145 150 155 160	

tgg ttc aac atc acc aac tgg ctg tgg tac atc aag att ttc atc atg

Trp Phe Asn Ile Thr Asn Trp Leu Trp Tyr Ile Lys Ile Phe Ile Met	528
165 170 175	

atc gtg ggc ggc ctg atc ggc ctg cgcc atc gtg ttc acc gtg ctg agc

Ile Val Gly Gly Leu Ile Gly Leu Arg Ile Val Phe Thr Val Leu Ser	576
180 185 190	

atc gtg aac cgc gtg cgcc cag ggc tac agc ccc ctg agc ttc cag acc

Ile Val Asn Arg Val Arg Gln Gly Tyr Ser Pro Leu Ser Phe Gln Thr	624
195 200 205	

cgc ctg ccc gtg ccc cgcc ggc ccc gac cgcc ccc gag ggc atc gag gag

Arg Leu Pro Val Pro Arg Gly Pro Asp Arg Pro Glu Gly Ile Glu Glu	672

210	215	220	
gag ggc ggc gag cgc gac cgc gac cgc acc cgc ctg gtg acc ggc Glu Gly Gly Glu Arg Asp Arg Asp Ser Thr Arg Leu Val Thr Gly			
225	230	235	720 240
ttc ctg ccc ctg atc tgg gac gac ctg cgc agc ctg ttc ctg ttc agc Phe Leu Pro Leu Ile Trp Asp Asp Leu Arg Ser Leu Phe Leu Phe Ser			
245	250	255	768
tac cat cga ttg cgc gac ctg ctg atc gtg gcc cgc atc gtg gag Tyr His Arg Leu Arg Asp Leu Leu Ile Val Ala Arg Ile Val Glu			
260	265	270	816
ctg ctg ggc cgg cgc ggc tgg gag atc ctg aag tac tgg tgg aac ctg Leu Leu Gly Arg Arg Gly Trp Glu Ile Leu Lys Tyr Trp Trp Asn Leu			
275	280	285	864
ctc cag tac tgg agc cag gag ctg aag aac tct gca gtg agc ctg ctg Leu Gln Tyr Trp Ser Gln Glu Leu Lys Asn Ser Ala Val Ser Leu Leu			
290	295	300	912
aac gcc acc gcc atc gcc gtg gcc gag ggc acc gac cgc gtg atc gag Asn Ala Thr Ala Ile Ala Val Ala Glu Gly Thr Asp Arg Val Ile Glu			
305	310	315	960 320
gtg gtg cag cgc atc tgg cgc ggc atc ctg cac atc ccc acc cga att Val Val Gln Arg Ile Trp Arg Gly Ile Leu His Ile Pro Thr Arg Ile			
325	330	335	1008
cgc cag ggc ttc gag cgc gcc ctg ctg taa Arg Gln Gly Phe Glu Arg Ala Leu Leu *			
340	345		1038

<210> 76
 <211> 345
 <212> PRT
 <213> Artificial Sequence

<400> 76			
Ala Val Gly Ile Gly Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly			
1	5	10	15
Ser Thr Met Gly Ala Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln			
20		25	30
Leu Leu Ser Gly Ile Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile			
35	40	45	
Glu Ala Gln Gln His Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln			
50	55	60	
Leu Gln Ala Arg Val Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg			
65	70	75	80
Phe Leu Gly Met Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala			
85	90	95	
Val Pro Trp Asn Ala Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp			
100	105	110	
Asp Asn Met Thr Trp Met Glu Trp Glu Arg Glu Ile Ser Asn Tyr Thr			
115	120	125	
Glu Ile Ile Tyr Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys			
130	135	140	

Asn Glu Leu Asp Leu Leu Gln Leu Asp Lys Trp Ala Ser Leu Trp Asn
145 150 155 160
Trp Phe Asn Ile Thr Asn Trp Leu Trp Tyr Ile Lys Ile Phe Ile Met
165 170 175
Ile Val Gly Gly Leu Ile Gly Leu Arg Ile Val Phe Thr Val Leu Ser
180 185 190
Ile Val Asn Arg Val Arg Gln Gly Tyr Ser Pro Leu Ser Phe Gln Thr
195 200 205
Arg Leu Pro Val Pro Arg Gly Pro Asp Arg Pro Glu Gly Ile Glu Glu
210 215 220
Glu Gly Gly Glu Arg Asp Arg Asp Arg Ser Thr Arg Leu Val Thr Gly
225 230 235 240
Phe Leu Pro Leu Ile Trp Asp Asp Leu Arg Ser Leu Phe Leu Phe Ser
245 250 255
Tyr His Arg Leu Arg Asp Leu Leu Ile Val Ala Arg Ile Val Glu
260 265 270
Leu Leu Gly Arg Arg Gly Trp Glu Ile Leu Lys Tyr Trp Trp Asn Leu
275 280 285
Leu Gln Tyr Trp Ser Gln Glu Leu Lys Asn Ser Ala Val Ser Leu Leu
290 295 300
Asn Ala Thr Ala Ile Ala Val Ala Glu Gly Thr Asp Arg Val Ile Glu
305 310 315 320
Val Val Gln Arg Ile Trp Arg Gly Ile Leu His Ile Pro Thr Arg Ile
325 330 335
Arg Gln Gly Phe Glu Arg Ala Leu Leu
340 345